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Symposium
on
Mammalian Genetics
and Reproduction

Contents

Introduction

Mammalian genetics, particularly the liation genetics of mice, has always been : of the major interests of the Biology rision of Oak Ridge National Laboray. So many basic findings have come of this field recently, both here and other laboratories, that it seemed timely hold a conference on Mammalian Gen:s and Reproduction and appropriate for to organize one.

The papers presented at the symposium ught out many points on basic problems l formed the focus for discussion of the sent status of the field as well as of :s of research for future development. As in previous symposia, free and open discussion was encouraged and is to a great part reprinted in this volume.

This conference, as well as all previous ones, was sponsored by the Biology Division of Oak Ridge National Laboratory in cooperation with the Division of Biology and Medicine of the Atomic Energy Commission. A committee under the chairmanship of Dr. W. L. Russell and consisting of Drs. Liane B. Russell, E. F. Oakberg and W. J. Welshons was responsible for arranging the program and also gave important editorial assistance during preparation of this volume.

Previous symposia in this series are:

1948—Radiation Genetics

1949—Radiation Microbiology and Biochemistry

1950—Biochemistry of Nucleic Acids

1951—Physiological Effects of Radiation at the Cellular Level

1952—Some Aspects of Microbial Metabolism

1953—Effects of Radiation and Other Deleterious Agents on Embryonic Development

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1957—Antibodies: Their Production and Mechanism of Action

1958—Genetic Approaches to Somatic Cell Variations

1959—Enzyme Reaction Mechanisms

ALEXANDER HOLLAENDER

nomalies of Fertilization Leading to Triploidy

C. R. AUSTIN
National Institute for Medical Research, Mill Hill, London, England

One of the commonest chromosomal errations arising spontaneously or ough experimental induction in mam-ls in triploidy. As a general rule, tri-id embryos in mammals are inviable, ough they may survive the first half pregnancy (Beatty, '57); if the fre-ency is sufficiently high, the condition n significantly reduce the fertility of the lividual. Triploidy necessarily involves incorporation in the embryo of three ploid sets of chromosomes, but there are eral ways in which it can come about. useful check-point at which classifica-n can be made is syngamy, when the nuclei come together and resolve into romosome groups that unite in the pro-ase of the first cleavage mitosis. The rpose of this paper is to classify the con-ions existing at syngamy that would d to triploidy in the embryo and to ex-ine systematically the mechanisms ough which they can arise.

In eggs destined to form triploid em-os, syngamy may involve any of the lowing combinations of pronuclei: one female and two male pronuclei (poly-andry), one male and two female pronuclei (polygyny), or a haploid pronucleus to-gether with a diploid pronucleus, either being the male and the other the female (aneugamy) (table 1).

POLYANDRY

The term polyandry denotes the pres-ence in an egg of more than one male pronucleus, in addition to the female pronucleus. There can be as many as 4 male pronuclei (Pikó, '58), but in the present context the term is applied only to eggs with two.

Cytology

The appearance of eggs in a state of polyandry has often been illustrated (e.g., Austin and Braden, '53; '55; Austin,'56d; Odor and Blandau, '56; Hamilton and Samuel, '56). In the rat egg, in which species the male pronucleus is known to be normally about twice the size of the female, the close similarity between the two male pronuclei is very evident. This

TABLE 1

The three forms of syngamy leading to triploidy and their principal modes of origin

Origin of anomaly	Normal syngamy	Forms of syngamy leading to triploidy		
		Polyandry	Polygyny	Aneugamy
Polyspermy	—	+[a]	—	—
Polar body suppression	—	—	+	—
Binuclear oocyte	—	—	*	—
Meiosis suppression	—	—	—	+
Octaploid oocyte	—	—	—	*
Diploid spermatozoon	—	—	—	*
Male pronuclei	●	● ●	●	● 2N ● or
Female pronuclei	●	●	● ●	● 2N

[a] Plus sign indicates the usual origin. Asterisk indicates the uncommon origin.

similarity is seen throughout pronuclear development and seems likely to be attributable to the influence of a correlating mechanism in the mammalian egg, such as has been observed also in sea urchin eggs (Brachet, '22). Eggs examined at later stages of fertilization show the presence of three more or less equal chromosome groups or of a single group that sometimes still bears evidence of being made up of three chromosome complements (this feature is illustrated in the review by Austin and Bishop, '57). Where the preparation has been suitable, it has been possible to establish that these single groups contain approximately a triploid number of chromosomes. Again, in suitably prepared eggs, the form of the first-cleavage spindle can be discerned and almost invariably this has been found to be bipolar (Austin and Braden, '53; Pikó, '58). Rat embryos examined up to the eleventh day of pregnancy exhibited triploidy at about the same frequency as that of the polyandry seen during fertilization (L. Pikó, personal communication, '59).

Origin

Polyandry arises from the entry of two spermatozoa into the vitellus of the egg (polyspermy) and their full participation in the process of fertilization. Recognition of polyandry involves a need to distinguish it from the other trinuclear state polygyny. In some animals, such as the rat, mouse, golden hamster, field vole, and pig, the presence of two sperm tails in the cytoplasm provides virtual proof of polyandry, and the greater size of the male pronucleus in the rat and mouse facilitates diagnosis in these animals. Where, however, sperm tails cannot be seen and the size relationships of the pronuclei are uncertain, dependence has to be placed upon less direct evidence. Male and female pronuclei may stain differently, as suggested by Pitkjanen ('55), Hamilton and Samuel ('56) and Thibault ('59), and this feature will be a most useful guide when its validity is fully established. The number of polar bodies is helpful, too, but owing to the variable persistence of the first polar body, the significance of this evidence must be determined specifically for each strain of animal used.

Throughout the animal kingdom, p andry is evidently an abnormal proc and there are broadly two means wher the egg is protected against its occurren prevention of entry of more than spermatozoon into the egg, and elim tion of supernumerary male pronuc These divisions correspond to Rothschi ('54) Type 1 and Type 2 inhibitions, spectively, and to the older classifica into pathological and physiological p spermy, respectively. The second eff elimination of pronuclei, may be achie either by suppression *in situ*, as in urode or by relegation to the periphery of blastodisc, as in birds, but is not kn to be brought about in any mamma egg. Mammals seem to depend exclusi on preventing the entry of extra s matozoa, but this should not be constr to mean that mammalian eggs have a single line of defense. They have, on contrary, defense in depth. There are less than four principal mechanisms wh can play a part in sperm exclusion, tho not all mammals show all of them:

1. Restriction in the number of s matozoa reaching the site of fertilizat A striking feature in the distributio spermatozoa in the female genital t after coitus is the great reduction in n ber that occurs as the spermatozoa proach the site of fertilization. Reduc seems to occur chiefly because the s matozoa have to traverse certain reg of the tract where the lumen narr abruptly, namely, the cervix (when ej lation is intravaginal), the uterine j tion, and isthmus of the Fallopian t Seemingly, too, the various physiolog features involved in sperm transpo muscular movements of the tract w vibratile activity of cilia, and the pro tion of secretions—all are adapted ap priately for highly selective transfer.

The chances of fertilization are natu related to the number of spermatozoa p ent about the eggs. It is consistent larger numbers are found in larger mals; in the rabbit, for example, 10 t times as many are found as in the with its much smaller site of fertiliza It has been calculated that rabbit an eggs experience about the same numb effective sperm collisions per unit of

ce area in a given time. In the rat, the mbers of spermatozoa passing into the gs are directly related to the numrs present at the site of fertilization able 2) (Braden and Austin, '54b). here was some suggestion, too, that the cidence of polyspermy could be similarly lated, but the figures were not significant. n rare occasions, however, very large mbers of spermatozoa are observed at e site of fertilization in rats mated under rmal circumstances, presumably because e controlling mechanism in the animal faulty. In these instances, several polyermic eggs may be recovered from the e animal, which is surely more than incidence (table 3).

2. *Protection by an outer coat.* Acmulated evidence shows that the jelly at of the sea urchin egg plays an imrtant role in protecting the egg against lyspermy (Hagström, '56). Spermatozoa em incapable of traversing the coat unded. The fertilizing spermatozoon bemes embedded in surface layers and nits a fine long process, the acrosome ament, that projects toward the vitellus nd penetrates the vitelline membrane. he spermatozoon, by now often immotile, then moves slowly inward, the nature of the movement suggesting that traction is being exerted upon the acrosome filament by the vitelline cytoplasm, which has become built up into a characteristic fertilization cone (Colwin and Colwin, '57). This reaction of the vitellus is normally evoked only by the first filament to make contact with it. If filaments from two spermatozoa make contact almost simultaneously, both spermatozoa may be engulfed and polyspermy ensue. But only some of the spermatozoa that contact the jelly surface become attached to it, and those that do vary in their rate of acrosome reaction and in the direction in which the filament is projected. The jelly coat can therefore be held to reduce greatly the number of potentially successful collisions of spermatozoa and egg and consequently the possibilities of polyspermy. Consistently, the incidence of polyspermy is much increased if the jelly coat is removed before the egg is placed in the sperm suspension.

In mammals, there is nothing precisely homologous with the jelly coat or acrosome reaction of the sea urchin, but the cumulus oophorus or granulosa-cell mass that surrounds the egg of many mammals at the

TABLE 2

The relation between the numbers of spermatozoa at the site of fertilization and the numbers entering the eggs

(From Braden and Austin, '54b)

Number of extra spermatozoa (within eggs) per tube	Mean number of spermatozoa per tube	Dispermic eggs
		%
0	35.7	0
1	39.4	1.7
2	44.1	1.5
3	56.3	2.4
4–5	65.5	
6–8	66.5	2.8
>8	136	

TABLE 3

The presence of polyspermic eggs associated with unusually large numbers of spermatozoa at the site of fertilization in a rat

No. of spermatozoa at site	Eggs					Polyspermic eggs
	Total	With 1 sperm	With 2 sperms	With 3 sperms	With 4 sperms	
341	6	1	3	0	2	1
477	5	0	4	1	0	2

time of fertilization may have some effects in common with those of the jelly coat. In particular, it may well impede the passage of spermatozoa, especially where the granulosa cells are most densely packed, namely, closely about the egg. There is some evidence that in rats and mice the density of packing of these "coronal" cells is sufficient to prevent fertilization from beginning for a measurable time after ovulation (Austin and Braden, '54c). It seems likely that a change in the cumulus involving some dispersal of the coronal cells, and taking 3–4 hours, is necessary before spermatozoa can gain access to rat and mouse eggs. The elimination of such a barrier in a gradual manner would certainly help to minimize the chances of successful sperm–egg collisions.

Mammalian eggs known to be surrounded by a broad layer of cumulus at the time of fertilization include those of the rodents, the rabbit, the pig, and the carnivores. On the other hand, in the ewe, cow, horse, and man, the cumulus breaks up early and spontaneously, and the egg is apparently denuded or nearly so when sperm penetration occurs.

3. The zona reaction. From a consideration of the numbers of spermatozoa entering the eggs of mice and rats, it was inferred that the zona pellucida undergoes a change after the entry of the first spermatozoon, tending to prevent the penetration of further spermatozoa (Braden *et al.*, '54). The reality of the change was supported by observations in mice and rats showing that enzymic digestion of the zona pellucida occurred less rapidly after sperm penetration (Smithberg, '52; Chang and Hunt, '56). The zona pellucida, therefore, could offer direct protection to the egg against polyspermy; and the means whereby the zona reaction was brought about was thought to be broadly analogous to the elevation of the fertilization membrane in sea urchin eggs (Austin and Braden, '56). In other words, attachment of the first fertilizing spermatozoon was believed to evoke in the vitelline cortex a response that was then propagated over the whole vitellus, the response being associated with the release of an agent that induced the zona reaction. Some of the evidence for this idea came from experiments in which a high body temperature was induced in mice immediately after coitus. Among the eggs recovered la there were a number in which spermato had entered the perivitelline space but not the vitellus and the number of spermato so involved was significantly greater th the number of spermatozoa in the perivi line space of eggs in which penetration the vitellus had taken place. Eviden the vitellus in the first group of eggs l been so affected by the heat treatment t attachment of and penetration by s matozoa was no longer possible; in same eggs, the zona reaction had fa and it seemed reasonable to infer t both effects could be ascribed to terioration of the vitellus. Further supp was gained by the finding that hams eggs, which develop a highly efficient z reaction, possess cortical granules these, like the cortical granules of urchin eggs, disappear after sperm atta ment (Austin, '56b). If the theory is rect, it may be necessary to suppose th in those mammalian eggs that do not play cortical granules yet have a zona action, an analogous though less str turally obvious mechanism exists.

Animals vary in the efficiency of th zona reactions. In the rat, two or m spermatozoa normally pass through zona pellucida of about 20% of eg which therefore have to fall back their last line of defense—the bl to polyspermy in the surface of the vi lus. The zona reaction in the rat accordingly be described as of moder efficiency, and this seems to be true in the mouse, the guinea pig, and so bats. By contrast, a highly efficient z reaction, rarely admitting the passage more than one spermatozoon, evide exists in the eggs of the golden hams the field vole, the dog, the cow and sheep. At the other extreme, a zona action seems to be altogether lacking in eggs of the rabbit, the pika, and possi the mole and the pocket gopher, for ma spermatozoa, often a hundred or so, n normally pass through the zona pellu in these animals.

4. The block to polyspermy. This the name given to the change that ta place in the vitelline surface as a result

attachment of the first spermatozoon; er the block has developed, attachment not possible with further spermatozoa. achment, of course, is the essential pre- e to sperm entry into the vitellus. The ck to polyspermy passes rapidly over vitelline cortex and it is estimated that, the sea urchin, complete protection is ieved in about 60 seconds (Rothschild,). So far, it has not been possible to ess the speed of the mammalian block polyspermy or to associate with it any ible change in the egg. The existence of block in mammalian eggs is known, refore, only through the accumulation the perivitelline space of spermatozoa t have been prevented from entering vitellus. When a zona reaction is lack- , the block to polyspermy is well in dence, as in the rabbit, and it is also arly in evidence in the eggs of the use and rat, which often contain sup- mentary spermatozoa in the perivitel- e space. In eggs such as those of the nster, however, spermatozoa are rarely ever found in the perivitelline space; ually the only eggs containing more n one spermatozoon are those that are yspermic. In this group, therefore, a ck to polyspermy may actually be lack- —or, equally, its efficiency is no higher n that of the zona reaction, so that it no obvious function.

Incidence

There are numerous reports on the ntaneous occurrence of three pronuclei eggs—they have been observed in the rat, mouse, hamster, field vole, rabbit, cat, ferret, pig, sheep and cow (see Austin and Walton, '60)—but not often was polygyny excluded or a sufficient number of eggs examined for a reliable expression of incidence. Instances in which the first requirement was properly met and 50 or more penetrated eggs recorded are set out in table 4. It can be seen that the spontaneous incidence in rodents of four species is around 1 or 2% of penetrated eggs. Differences between groups of animals within the same species are as large as differences between species, despite the dependence of different species (for example, the rat and mouse on the one hand and the hamster on the other) on different devices for protection against polyspermy. Differences between inbred strains are particularly striking, as reported by Pikó ('58) for two strains of rats tested under the same conditions and mated with males of the same stock ("Jouy"). Susceptibility to polyspermy is evidently to some extent under gene control, and in this matter it is the genotype of the female that is important. The particular line of defense influenced is not known for certain.

Much larger variations in incidence can be induced by experiment. Thus, when rats were not allowed coitus until nearly the end of estrus (delayed mating), the proportion of polyspermic eggs was increased in varying degrees up to elevenfold (table 5). Here too, presumably, gene-controlled strain and stock differences played a part: this is certainly shown by the results reported by Braden and by

TABLE 4

Incidence of polyandry based on observations in which the presence of two sperm tails aided recognition of the anomaly

Animal	No. of penetrated eggs	No. of polyspermic eggs	Incidence	Reference
			%	
Rat	810	10	1.2	Austin and Braden, '53
Rat	326	6	1.8	Austin, '56c
Rat	336	1	0.3	Odor and Blandau, '56
Rat[a]	165	0	0	Pikó, '58
Rat[b]	126	4	3.2	
Rat[c]	114	1	0.9	
Mouse	169	2	1.2	Braden *et al.*, '54
Mouse	3377	30	0.9	Braden, '57
Hamster	725	10	1.4	Austin and Braden, '56
Field vole	59	1	2	Austin, '57

[a,b,c] Stock "Jouy" and inbred strains WAG and Wistar CF, respectively.

TABLE 5

Effect of delayed mating and of strain of animal on the incidence of polyandry in rats

Rat stock used		With normal mating	With delayed mating	Increase	Reference
Female	Male				
		%[a]	%[a]	*times*	
Outbred albino		1.2	8.2	7	Austin and Braden, '53
Outbred albino		1.8	3.3	Not significant	Austin, '56c
Wistar albino		0.3	3.3	11	Odor and Blandau, '56
WAG	WAG	—	9.2	—	Braden, '58
WAG	PVG	—	7.6	—	
PVG	PVG	—	4.3	—	
PVG	WAG	—	3.1	—	
F_1	WAG	—	2.5	—	
"Jouy"	"Jouy"	0.0	4.5	Varies	Pikó, '58
WAG	"Jouy"	3.2	6.6	2	
Wistar CF	"Jouy"	0.9	7.1	8	

[a] Eggs in polyandry as percentage of total penetrated eggs.

Pikó. The incidence after delayed mating is attributable chiefly to aging of the eggs leading to loss of efficiency in both the block to polyspermy and the zona reaction (Austin and Braden, '53). The change is reminiscent of the increase in the frequency of polyspermy found in sea urchin eggs that have been allowed to become "stale" before treatment with sperm suspension. Other factors may also be involved: the protective property of the cumulus may well depreciate, since the follicle cells tend to lose their dense aggregation and migrate out of the matrix as time passes (Blandau, '60); control of sperm number by the female tract evidently relaxes late in estrus, at least in the rat (Braden and Austin, '54b).

Some observation have also been made with rabbits: in a stock of outbred white rabbits, with normal mating the incidence of trinuclear eggs was in the region of 1%, but mating at the time of induced ovulation caused an increase to about 16% (Austin and Braden, '53). It was pointed out, however, that, since the sperm tail is rarely distinguishable in the cytoplasm of the rabbit egg, positive identification of polyspermy could not be made. In about half the trinuclear eggs, two pronuclei were large and one small; in the remainder, two pronuclei were small and one large. The suggestion was therefore made that the eggs with two large pronuclei were probably polyspermic and the others could have been attributable to the monospermic fertilization of eggs containing two female pronuclei (i.e., polygyny). problem has been reinvestigated by ficially inseminating rabbits at var times at or after the induction of ovula (table 6). The eggs were examined in fresh state with varying degrees of c pression under a coverglass then fixe *situ* and stained for chromatin com ents. Anomalies of fertilization were only in rabbits inseminated after ov tion, the chief irregularity being the nuclear state (fig. 1). Study of the nuclear eggs confirmed earlier suspic that pronuclear size in rabbit eggs is a reliable means of identification; in r of the eggs, one pronucleus was m larger than the other, but in some eggs pronuclei were about the same size. In of the trinuclear eggs, two sperm could be discerned in the cytoplasm; lay near and could have been attache pronuclei, and these pronuclei were a little smaller than the third pronucl The suggestion is therefore that, in ra eggs, unlike those of the rat and mo the male pronucleus is the smaller of two. The other trinuclear egg with polar bodies had two large pronuclei one small one; it is, however, regarde probably exhibiting polyandry becaus had two polar bodies. The numbe polar bodies seemed a safer criterion s very nearly all the binuclear eggs had Accordingly, the remaining four trinuc eggs, which had one or no polar bo were considered to be polygynic.

TABLE 6

Pronuclei and polar bodies in eggs from rabbits inseminated at different times with respect to ovulation

Time of ovulation taken to be 10 hours after intravenous injection of human chorionic gonadotrophin.

	Hours before (—) or after (+) ovulation					
	—10	—2	0	+1	+2	+3
No. of rabbits	5	1	2	6	3	2
Total eggs	33	5	14	46	16	10
Penetrated eggs	31	4	7	38[a]	4	0
Binuclear eggs 1 Pb	3	0	0	1	0	0
Binuclear eggs 2 Pb	28	4	7	31	2	0
Trinuclear eggs 0 Pb	0	0	0	1	0	0
Trinuclear eggs 1 Pb	0	0	0	1	2	0
Trinuclear eggs 2 Pb	0	0	0	2[b]	0	0

[a] Figure includes an egg with five nuclei of various sizes and a binuclear egg with three apparent polar bodies.

[b] Two sperm tails identified in one trinuclear egg.

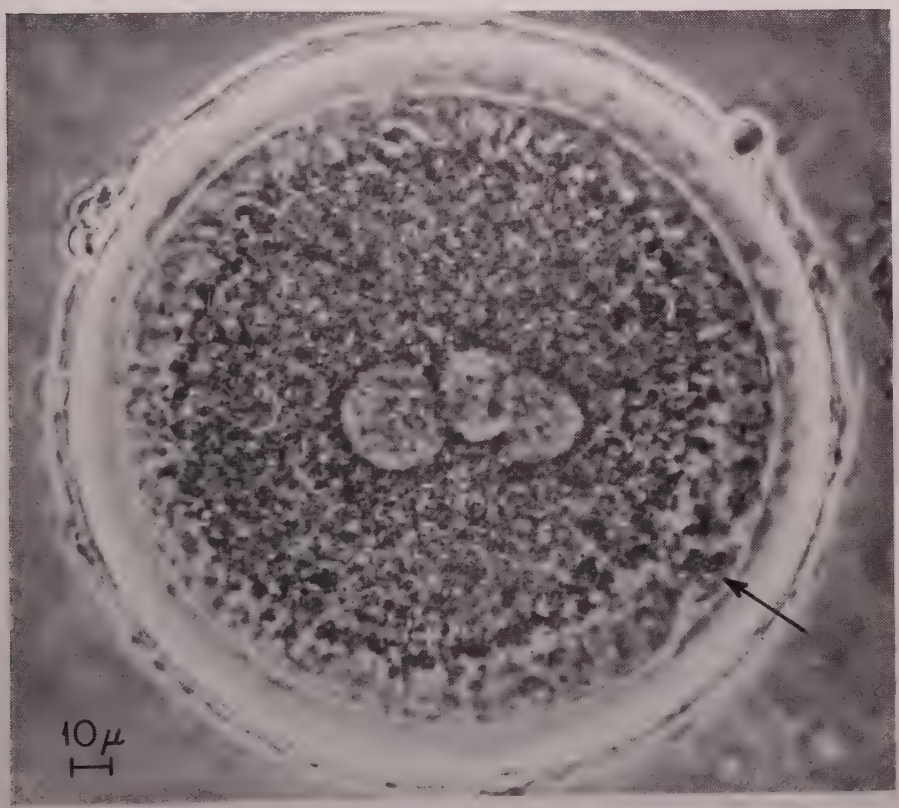

Fig. 1 A trinuclear egg with a single polar body (arrow), from a rabbit inseminated about 1 hour after ovulation.

combined incidence of the two anomalies was about 12%.

In the pig, too, the frequency of occurrence of trinuclear eggs can be greatly increased by the fertilization of aging eggs. Hancock ('59) found no instances among 34 eggs recovered from sows mated at the start of estrus, but when mating took place 24, 30, and 48 hours after the onset of estrus 1/29, 7/56 and 12/29, respectively, were trinuclear. Two sperm tails were occasionally identified, and in several of the trinuclear eggs two polar bodies could be distinguished; Hancock therefore believes that polyspermy was a major cause but cautions that this may not have accounted for all cases. Thibault ('59) reported only one polyspermic egg among 55 recovered from sows mated within 36 hours after the start of estrus; animals mated later yielded six polyspermic egg out of 53 (11%). On the other hand, 11 eggs (21%) exhibited polygyny. The combined incidence of trinuclear eggs in late-mated pigs was thus about 30%.

Elevation of temperature, either by local application of warm water to the Fallopian tube or by the induction of hyperthermia can lead to large increases in the frequency of polyspermy in rats, the level rising to as high as 34% (table 7).

POLYGYNY

As with polyandry, the term polygyny can be used to denote the presence in eggs of any number of pronuclei in excess of one—female pronuclei in this case, of course. In the present context, however, the term is intended to imply only syngamy between two female pronuclei and a male pronucleus.

Cytology

The appearance of rat and mouse eggs exhibiting polygyny has been illustrated in several papers (Austin and Braden, '54a; Braden, '57) and pictures of pig eggs in this condition appear in Thibault's report ('59). The problem of recognizing polygyny, which chiefly involves distinguishing it from polyandry, has already been discussed. Direct cytological evidence on the terminal stages of fertilization in polygyny is lacking, but the great body of indirect evidence leaves no doubt that, as in polyandry, the three pronuclei come together at syngamy and resolve into chromosome groups that unite in the prophase of the first cleavage division.

Origin

Binuclear oocyte. Early primary oocytes bearing two nuclei have often been reported; they are associated almost exclusively with immature animals and there appears to be general agreement that they are abortive and rarely if ever reach full development and undergo maturation (see Austin and Walton, '60). There is, however, a chance that an occasional binuclear oocyte, developing in an adult animal, may survive to maturation and become fertilized. They are thought to be formed either by nuclear division in an oogonium that then fails to undergo cytoplasmic cleavage or by cytoplasmic fusion of two oogonia. These are also the ways in which giant eggs are believed to arise, and it may well be that the binuclear oocytes recorded in the literature were in fact giant eggs in the making; since many were seen in the growing phase, their full usual size would not have been apparent. It is therefore suggested that binuclear giant eggs sometimes ovulated in adult laboratory animals represent the occasional binuclear oocytes that survive. They have been seen as secondary oocytes with two second maturation spindles (Aus-

TABLE 7

Increase in the frequency of polyspermy in rats as a result of delayed mating and heat treatment

Mating	Treatment	Incidence	Reference
		%	
Normal	None	1.2	Austin and Braden, '53
Delayed	None	8	
Delayed	Local heat	16	Austin and Braden, '54b
Delayed	Hyperthermia (mature rats)	34	Austin, '56c

d Bishop, '57) and as pronuclear eggs th a male and two female pronuclei (Aus- n and Braden, '54a). Normal-looking two- ll giant eggs have also been recovered, ch as those of the rat and cotton rat ustin and Amoroso, '59).

Giant eggs have long been known in n-mammals, and it has been shown that ey are capable of undergoing fertiliza- n and developing into fully viable young. cording to their supposed manner of rmation, giant eggs can be expected to be taploid as fully grown primary oocytes (8 by DNA content, but chromosomes still ganized at 4 N), and diploid after chro- osome reduction; consistently, sea urchin ant eggs give rise to triploid embryos ter fertilization (Wilson, '28, p. 972). me sea urchin giant eggs are uninu- ear, possibly because of nuclear as well cytoplasmic oogonial fusion. This is al- true of some mammalian examples— fertilization, these would present one rm of aneugamy, which is considered ter.

Polar body suppression. Suppression of her the first or second polar body, as- ciated with completion of both meiotic visions, can result in polygyny. The way which suppression can come about, involving movement of the spindle inward and away from the egg surface, has been clearly demonstrated by Fankhauser and Godwin's work on *Triturus* ('48). The formation of two early female pronuclei from the second polar spindle in a rat egg has been studied *in vitro*, when compression of the egg beneath the coverslip inhibited extrusion of the polar body (Austin, '51). Spontaneous formation of two pronuclei in unfertilized hamster eggs held *in vitro* has also been observed (fig. 2). Hamster eggs are evidently rather prone to do this (Austin, '56a). Although there is no doubt that nucleus formation from both chromosome groups of the telophase meiotic spindle readily occurs, it seems unlikely that this follows merely the submergence of the spindle, as in the urodele egg. Rodent eggs seem to divide very easily, as witness the early fragmentation of unfertilized rat eggs, and it is known from the occurrence of "immediate cleavage" that the meiotic spindle is quite capable of dividing the mouse egg into two approximately equal parts (Braden and Austin, '54c). We must therefore suppose that, when two female pronuclei develop, the mechanism responsible for expulsion of the polar body has been

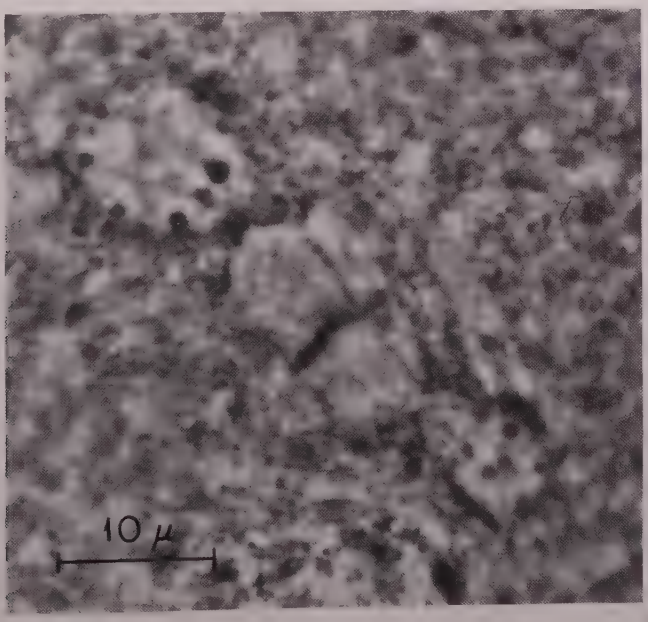

Fig. 2 Early development of two female pronuclei after second polar body suppression in a hamster egg.

inhibited, but it is unnecessary to postulate spindle submergence, which may not in fact occur.

Loss of the capacity to produce a polar body is to some extent gene conditioned; as Braden ('57) has demonstrated, it is the genotype of the female that is important and inhibition nearly always involves the second polar body. Suppression of the second polar body was also found to be the main cytological anomaly produced by subjecting mouse eggs to heat treatment (Beatty and Fischberg, '49; Fischberg and Beatty, '50, '52; Braden and Austin, '54a). There is a distinct species difference here, for rat eggs similarly treated suffered only temporary suppression of the polar body and showed instead, as already mentioned, a heightened incidence of polyspermy. It is not unreasonable to find these two cytological changes induced by the same agent for both are functionally dependent on properties of the vitelline surface. Consistently, aging of the egg is another factor affecting both phenomena, which was well shown by the data described in connection with polyandry.

Incidence

In outbred stocks of rats and mice, somewhat less than 0.1% of eggs were normally found in polygyny (Austin and Braden, '54a). Triploidy ascribed to polar body suppression in "nonsilver" strains of mice was about 0.25%; higher levels were met with when mice of the "silver" stocks were tested, the greatest frequency occurring when silver females were mated with nonsilver (CBA) males: 5.7% (Beatty, '57).

The reported incidence of giant eggs is in the range of 0.1 to 0.5% in rats, mice and rabbits (Austin and Braden, '54a). Kent's work ('59) suggests that it may be possible to influence the frequency of binuclear (= giant?) oocytes in hamsters by estrogen treatment.

Heat treatment of mouse eggs gave 11% triploidy at 3.5 days gestation, and colchicine treatment was about equally successful (Beatty, '57; Edwards, '58).

The highest incidence of polygyny is seen as the result of delayed mating or insemination in some groups of rabbits and pigs, where the rate may exceed 20% and surpass that of polyspermy. These points have been discussed in the section polyspermy.

ANEUGAMY

Cytology

Since aneugamy by definition signif syngamy between a diploid female p nucleus and a haploid male pronucle or a haploid female and a diploid ma diagnosis would require a knowledge the ploidy of each pronucleus. It is p sible, of course, that the ratio betwe the volumes of the two pronuclei will diff from normal if one of them is diploid. cause of the competition between pronuc (Austin and Braden, '55), the haplo pronucleus would be likely to assume smaller size than normal, and this wou tend to accentuate the altered ratio. In t rat, the average normal ratio of female male pronuclear volumes is about 1:2 If the male pronucleus were diploid, t ratio would presumably be wider than th whereas, if the female were diploid, a proximate equality of pronuclear volum would be expected (the effect is illu trated diagrammatically in table 1). H the normal range of ratios is already wide that the alteration might well go u noticed. Thus, in a series of 28 rat eg taken at random under normal condition the ratios of the volumes of the pronuc varied from 1:1.5 to 1:5. It seems, the fore, that evidence for the diagnosis aneugamy would have to be obtained fr counts of chromosomes in the two grou into which the pronuclei resolve near t close of fertilization, and so far this d not seem to have been done.

Origin

Octaploid primary oocyte. An egg c taining a diploid female pronucleus cou develop from a uninuclear primary oocy that was octaploid (in respect of ch matids and DNA content) just before duction, and this in turn could arise, much the same way as with binucle (and giant) eggs, through the suppressi of an oogonial division after chromoso replication or through complete fusi of oogonia. As already noted, giant eg are sometimes uninuclear. An indicati that such eggs contain twice the norm chromosome complement is provided by t

ding of a rat giant egg undergoing dimentary parthenogenesis; the nucleo- volume in this egg was twice that of rmal-sized eggs in a similar state (Aus- and Braden, '55). The fact that the clear volume was not appreciably larger an those of normal-sized early parthenoetic eggs is not necessarily an objection this argument, for nuclear size is more nited by substrate supply than is nucleo- volume.

Meiotic suppression. If either the first second meiotic division is suppressed *fore* anaphase separation of the chroosomes, a diploid female pronucleus uld result. From a purely cytodynamic int of view, this seems just as likely to ppen as polar body inhibition *after* anaase separation, yielding two female proclei. In artificially activated unfertilized bbit eggs, Thibault ('49) reported supession of the second meiotic division d development of a single, presumably ploid, nucleus. He considered such eggs ore likely to begin embryonic developent than those in which inhibition of lar body formation, but not of meiosis, ads to two haploid nuclei. Accordingly, eugamy, which would be the amphimic- equivalent of mononuclear parthenonesis as shown by the first type of eggs, ay well be a significant source of triploidy in the rabbit. On the other hand, aden's observations ('57) show that in ice polygyny occurs with sufficient freency to account fully for the incidence triploidy recorded in 3.5-day embryos. contrast, in rats treated with colchicine, out 90% of normal-looking pronuclear gs had no second polar body and could ell have possessed diploid female proclei (Austin and Braden, '54b). Subct to fuller analysis, this finding cerinly suggests that aneugamy can be adily induced in rat eggs.

Diploid spermatozoon. The third clearly ssible origin of aneugamy is the fertiliion of a normal egg with a diploid speratozoon, which could be expected to rm a diploid male pronucleus. Theoretally, diploid spermatozoa could be deved through failure of a spermatogonial vision after chromosome replication, fuon of spermatogonia, or suppression of her the first or the second meiotic divisions in spermatogenesis. However, no well-established instances of anomalies of these kinds seem to have been recorded in mammals, as spontaneous phenomena, and the effects of heat and colchicine treatment have yet to be properly elucidated. Occasionally spermatozoa are found that are much larger than normal and that could be polyploid. Instances have been seen among spermatozoa from a cat; in the semen of this animal, which was known to be fertile, roughly 5% of the cells were unusually large (unpublished data of M. W. H. Bishop and C. R. Austin). The stage of spermatogenesis at which the fault occurs in this case has not yet been determined.

Incidence

There seems to be virtually no precise evidence on the incidence of aneugamy. Among more than 1500 mouse oocytes, Pesonen ('46a, b) found one with a tetraploid second-maturation spindle, which could have given rise to a diploid female pronucleus; the extreme rarity thus suggested is consistent with Braden's findings ('57). In a series of more than 7000 rat eggs, Austin and Braden ('54a) noted four giant eggs with well-formed male and female pronuclei; they could have been examples of aneugamy.

CONCLUSIONS

Triploid mammalian embryos may develop from eggs in which fertilization is abnormal through the occurrence of one or other of three anomalous forms of syngamy—polyandry, polygyny, and aneugamy.

Polyandry arises from polyspermy, against which the mammalian egg has four main lines of defense: restriction of numbers of spermatozoa reaching the site of fertilization, impedance of sperm penetration by the cumulus oophorus, the zona reaction, and the block to polyspermy. The efficiency of all these mechanisms varies with the genetic constitution of the animal and can also be influenced by experimental means.

Polygyny, in its triploid form, involves syngamy between a male and two female pronuclei and derives from the fertilization of a binuclear oocyte, which is

sometimes a giant oocyte, or of an egg in which either the first or the second polar body has been suppressed. The commonest pathway is through second polar body suppression. Polygyny, like polyandry, is under some genic control and can be induced experimentally.

Aneugamy is attributable to fertilization of an egg deriving from an octaploid late primary oocyte or through suppression of the first or second meiotic division, or the fertilization of a normal egg by a diploid spermatozoon. Although the mechanisms by which aneugamy could arise appear to exist in mammals, there is no direct evidence of its occurrence or incidence—it seems to be very rare.

The most important sources of triploidy are therefore polyandry and polygyny, the latter being attributable to second polar body suppression. The incidence of both these classes of anomaly is greatly increased in aging eggs—polyandry almost exclusively in rats, polygyny chiefly but not exclusively in mice, and a varying balance of the two phenomena in rabbits and pigs. The combined incidence of the two anomalies may surpass 25% and even approach 50% of eggs undergoing fertilization.

OPEN DISCUSSION

POPP[1]: Apparently, the X and Y chromosome differences are not good enough to detect the difference between male and female pronuclei, or have you examined them for this? At least in some animals, such as the rabbit, one might be able to use X and Y chromosome markers.

AUSTIN: This would only distinguish between different male pronuclei.

POPP: Yes. If you had one female pronucleus and two male pronuclei, could you distinguish them by the difference in the X and Y?

AUSTIN: We have entertained the thought that something similar to sex chromatin might exist in male pronuclei containing the X chromosome and be lacking from those containing the Y, but despite a long and careful study of rat eggs I have been quite unable to find such a distinguishing structure.

POPP: Has anyone attempted to culture these triploid cells to determine whether they are viable in a tissue culture?

AUSTIN: Not to my knowledge. Th embryos are known to be developing w at 9½ and 11 days of gestation in mi and rats, respectively. They are a l smaller than normal, but they are st growing quite nicely; so it should be po sible.

STERN[2]: What happens to the centr somes brought in by the two spermatozo Do they not form triplolar spindles?

AUSTIN: This is the unique feature polyspermy in mammalian eggs: the spi dle is almost always bipolar. We ha seen a single tripolar spindle, but it w clearly abnormal. Otherwise, we ha regularly found bipolar spindles. I do n know what happens to the sperm centr somes.

WAELSCH[3]: Do you have any idea wh these animals die at the time they do, i. at 9½ or 11 days?

AUSTIN: None at all. This is a proble for the geneticists.

WAELSCH: I was wondering of possibl morphological or physiological symptom preceding death.

AUSTIN: There are no observations my knowledge.

LINDSLEY[4]: In *Drosophila* we occasio ally see evidence of fusion of the e pronucleus with one sperm nucleus an of one of the polar body nuclei with a other sperm nucleus to produce two f sion nuclei of different genotype. Suc instances develop into mosaics. Have yo any evidence that this can ever happen mammals?

AUSTIN: I don't think it has been r ported in mammals. We do have instanc in which polygyny and polyandry occur one egg. You see four pronuclei, two ma and two female. Their behavior, in cluste ing together intimately, suggests that yo would get syngamy followed by the fo mation of a bipolar spindle again, but th possibilities of further development ar unknown.

LINDSLEY: It is my impression that th ploids in *Drosophila* generally arise fro fusion of a haploid and a diploid prom

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[1] R. A. Popp, Oak Ridge National Laborato
[2] Curt Stern, University of California, Berkel
[3] Salome G. Waelsch, Albert Einstein Colle of Medicine.
[4] D. L. Lindsley, Oak Ridge National Laborato

s and not from the fusion of three oid pronuclei.

RADEN[5]: I think it should be pointed that, as far as is known, a zygote nus is not formed in mammalian eggs; gamy of the haploid chromosome ips occurs. Mosaic eggs may possibly e from "immediate cleavage," that is, egg dividing into halves at a maturadivision. It is possible that those two es could be fertilized by different m, resulting in mosaic.

OLLANDER[6]: I have not looked at them n the point of view of polyploidy. Any, since they are not mammals, I think ad better drop them.

LDEN[7]: I got the impression that you ght the change on the egg surface presumably prevents polyspermy was h slower than had been heretofore. ch of the animals was heated and ch was last spermed—the male or the ale?

USTIN: In answer to the first ques: I was referring to the zona reaction ne rat, which we estimate to take some- between 10 minutes and 2 hours. the rat is one of those beasts in which endence is placed on both the zona tion and the block to polyspermy. So sperm that the zona reaction misses, block to polyspermy will pick up. in in the rat there is a massive cumulus this may well impede the approach ome sperm. So the rat egg is pretty protected even though the zona reon is apparently slow. In other ani-s, such as the hamster, the zona tion is evidently very fast indeed and umably is the chief protection against spermy. In answer to the second ques: the animal heated was the female, mating.

HAIRMAN DUNN[8]: Dr. Austin, did you ibe the difference in size of the treated nals to differences in age? Is it to be rred that the older animals responded higher rate to the effect of heat?

USTIN: It just seems that the aging of egg, which can take place when mating elayed, is faster in the older animals.

OLFF[9]: I noticed, on your slide showhyperthermia, that you had fewer eggs heating. Is this just chance sample size or does hyperthermia do something so that fewer eggs are produced?

AUSTIN: It was just sample size.

GREEN[10]: Do you know whether the triploidy that occurs in the silver stock is an effect of the silver gene?

AUSTIN: I would like to refer that question to Dr. Braden, who was actively connected with that work.

BRADEN: The evidence is that it is not directly connected with the silver gene (Braden, '57).

MINTZ[11]: "Overripeness" of amphibian eggs is known to lead to certain kinds of anomalies; might not the situation in the mammal be a comparable one?

AUSTIN: That is in fact what we believe to be the consequence of delayed mating. I think that aging of the egg or overripeness is the reason for the higher incidence of polyspermy with delayed mating.

STERN: You suggested that we go back to the geneticists as to why the triploids do not develop. There is no obvious genetic cause for this failure. Could it be that the interaction of the fetal tissues with those of the mother depend on both being diploid and that metabolic transfer between the cells of a triploid zygote and a diploid uterus is inadequate? This suggestion derives from some cases of insufficient interaction in higher plants. There, usually, the embryo is diploid and the endosperm triploid, a relation that permits normal development. However, in hybrids between individuals of different degrees of ploidy, situations arise in which the ploidy ratio between embryo and endosperm differs from the normal 2:3, with the result that the embryo may fail to develop.

WAELSCH: If they die at 9½ days, it would be at a time when really the period of interchange through the trophoblast would be at its end; the embryo should

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[5] A. W. H. Braden, Commonwealth Scientific and Industrial Research Organization, Prospect, New South Wales.

[6] W. F. Hollander, Iowa State University.

[7] R. H. Alden, University of Tennessee, Memphis.

[8] L. C. Dunn, Columbia University.

[9] S. Wolff, Oak Ridge National Laboratory.

[10] Margaret Green, Roscoe B. Jackson Memorial Laboratory.

[11] Beatrice Mintz, The Institute for Cancer Research, Philadelphia.

diet earlier, if this interchange is disturbed, because at 9½ days, the embryo has a perfectly good placenta. So by that time the problem of nutrient exchange through the trophoblast should have been overcome. On the other hand, placenta formation itself may be abnormal owing to the mechanism that you suggest. This is why I would be happy to see some observations made on these embryos just before they die.

RUNNER[12]: The origin of triploidy in mammals has been ingeniously demonstrated by Dr. Austin, and apparently the state of triploidy is invariably lethal. Concerning the cause of death of the triploid embryos, perhaps the possibility should not be overlooked that in these embryos death may result from abnormal nucleic acid metabolism (e.g., excessive DPN production) rather than from mechanical problems of the chromosomes or presence of deleterious genes in excess. If so, onset of death would occur with the appearance of a specific phase of ontogeny sensitive to the unusual nucleic acid metabolism—the ambush effect in embryology. For example, exposure of cells to unusual RNA (Niu, Hiashi) or the presence of supernumerary chromosomes (mongolism, Klinefelter's syndrome, Turner's syndrome) is associated with specific developmental changes aside from exaggerated manifestations of any known mutants. Even the nucleus from the egg of a frog introduced into the cavity of a blastocoel of the frog embryo causes derangement in development (Huff). Something introduced with accessory chromosomes or some products of the chromosomes can interfere with development.

It seems useful and necessary at our present state of knowledge to attempt to distinguish between the possibilities of death associated with presence of specific mutant genes and death associated with generalized derangement of nucleic acid metabolism.

BATEMAN[13]: Have you any evidence about the origin of the centrosomes in normal cleavage, whether they were both maternal or one is contributed by the male nucleus? I am concerned about this question of the normal bipolar spindle, whether there are two or three pronuclei.

AUSTIN: The sperm is believed to introduce a centrosome at fertilization, whether this is normally responsible the first cleavage spindle has not b established. Certainly, the evidence that trinuclear mammalian eggs, whet polyandric or polygynic, can enter u normal cleavage. And this suggests t in mammals, there is some kind of reg tory mechanism that ensures the for tion of a bipolar spindle, possibly by s pression of supernumerary centrosom

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enetic Influences on the Morphology and Function f the Gametes

A. W. H. BRADEN
Division of Animal Physiology, C.S.I.R.O., Ian Clunies Ross Animal Research Laboratory, Prospect, N.S.W., Australia

In the past there has been a tendency in ammalian genetics to assume that the orphology and reactions of the gametes ere independent of their specific gene ntent, being rather determined by the ereductional genetic composition and by e soma. In plants there is ample evi- nce that genes may function in the metes (Sturtevant and Beadle, '40), but neticists have generally held, with Mul- r and Settles ('27), that this is not the se in animals. For selection to operate the gametes (in the haploid phase) ere must be phenotypic variation be- een gametes from an individual animal. ne latter is a *sine qua non* for selective rtilization when a single pair of animals used. Evidence for selective fertiliza- on in mammals has largely been derived om matings of one female with two dif- rent males (King, '29; Cole and Davis, 4) or by artificial insemination with se- en from two or more males (Edwards, 5; Beatty, '57, '60). Selective fertiliza- on when a single male is used involves a parture from normal Mendelian ratios r individual genes and herein lies the ason why genetic influences on the gam- es have been largely discounted. The eat majority of genes that have been udied in animals have been shown to be ood" genes, that is, they segregate in the pected 1:1 fashion. Moreover, the aber- nt segregations of "bad" genes can usu- y be accounted for in terms of pheno- pic overlapping in the zygotes, differen- l viability or, occasionally, abnormalities meiosis. There may be some bias in is situation, at least for mouse genetics, r in laboratories in which the mouse is ed, more apparent mutations occur than ere are opportunities to investigate, with e result that often only the "good" genes are chosen for study. Nevertheless, there is a "bad" gene in the mouse that has been shown to affect the function of the spermatozoa, namely the multiallelic *T* locus. The first reports of this locus were by Dobrovolskaia-Zawadskaia and Kobozieff, ('32) and Chesley and Dunn, ('36). One of the mutant alleles that occurs at this locus, Brachyury (*T*), differs from all the others in that it has a dominant effect on tail length and its segregation ratio is normal. A large number of other mutant alleles, symbolized by *t* with a superscript (e.g., t^0, t^{w11}), have been reported. They are characterized by the production of taillessness when in conjunction with *T*, and by aberrant paternal segregation (or, better, transmission) ratios. Homozygotes of *T* and many *t* alleles die *in utero*. In spite of this, *t* alleles have been found, usually in high frequency, in almost every wild population of mice studied both in the United States and Japan (Dunn, '55; Tutikawa, '55). Before going on to discuss the manifestations of this fascinating locus, a digression on the normal physiology of reproduction in the mouse and a review of interstrain variation in gametic phenotype is called for.

TIME RELATIONS OF EVENTS CONNECTED WITH FERTILIZATION IN THE MOUSE

In the mouse, as in the rat (Austin and Braden, '54) and hamster (Austin, '56), the time of ovulation is closely linked with the diurnal rhythm of light and darkness (Snell *et al.*, '40; Braden and Austin, '54a, Braden, '57a). Ovulation is the major event in the estrous cycle and other events such as coitus, meiosis, and spermatozoon penetration are best related to it. In mice kept under the normal daily rhythm of illumi-

nation, ovulation occurs between about midnight and 8 A.M., but the length of this period varies with both the strain of mice and the length of the period of darkness (Braden, '57a). In any one mouse, ovulation was estimated to occupy one-half to three quarters of an hour. This indicates that the relatively long interval required for ovulation in a group of mice is largely the result of interanimal variation in the onset of ovulation. Coitus normally takes place before ovulation, the interval between the two events having been found to be 1–5 hours in various stocks of mice. If there is no opportunity for mating earlier, female mice will accept the male for up to 8 hours after ovulation.

Accurate timing of meiosis is important in investigations such as that on radiation of mouse oocytes. In adult mice in which ovulation was induced by gonadotrophin injections, the first meiosis began 9 hours before ovulation and reached completion with the extrusion of the first polar body just before ovulation (Edwards and Gates, '59). Metaphase occupied 6.0 ± 0.3 hours, anaphase 1.2 ± 0.3 hours, and telophase 0.3 ± 0.2 hours. The second meiosis begins immediately after the completion of the first meiosis but is arrested at metaphase and does not normally proceed until spermatozoon penetration of the egg has occurred.

In female mice that ovulate naturally and copulate before or at about the time of ovulation, there is usually an interval of several hours before penetration of the eggs begins. The mean interval in any one mouse was estimated to be 2½–3 hours. A further interval of 4 hours normally elapses before spermatozoon penetration of all the eggs in a mouse is complete (Braden and Austin, '54a). Both these intervals are considerably briefer in adult mice in which ovulation has been induced by gonadotrophin injections (Edwards and Gates, '59; Braden, '59a). The delay between ovulation and penetration in untreated mice is not because the spermatozoa do not reach the vicinity of the eggs early, but apparently because the egg membranes, in particular the cumulus oophorus, need some form of maturation. This phenomenon has been observed only in two other species (rat—Austin a Braden, '54; hamster—Austin, '56; Strau '56). From a study of inbred strains mice and their crosses, it appears that genotype of the female may markedly fluence the extent of the interval betwe ovulation and penetration (Braden, '58

From data on the mean number of sp matozoa that were present in penetra eggs when 40–60% of the eggs in a one Fallopian tube had been penetrat the mean number of eggs per tube, a an estimate of the mean time required all, or nearly all, the eggs in a tube to penetrated, it is possible to estimate frequency of successful spermatozoon–e collisions. For mice that mated at the n mal time in relation to ovulation, the e mated frequency was one successful co sion in every 20–30 minutes. In mice ma about 4 hours after ovulation, the rate w estimated to be one per 12–17 minut Estimates for the rates in rats and rabb have been given elsewhere (Braden a Austin, '54b).

By manipulating the time of coitus relation to ovulation, it is possible to m ify the length of time that spermato spend in the female tract before pe tration occurs, as well as altering the sp matozoon—egg collision rate. In m mated at the normal time, the average terval between coitus and spermatozo penetration of the eggs was about 8 hou whereas in late-mated mice it was abou hours (Braden and Austin, '54a).

INTERSTRAIN VARIATION IN GAMETIC PHENOTYPE

Variation between strains of mice the characteristic morphology of the ga etes or in their function provides evide that hereditary factors may express the selves in the phenotype of the gamet These factors may act indirectly throu the soma of the male or female, or direc in the gamete itself.

Investigation of a number of inb strains of mice revealed characteristic terstrain differences in the shape and av age dimensions of the spermatozoon he (Braden, '59b; Beatty and Sharma, ' Sharma, '60). Antigenic differences tween the spermatozoa of three inb

ins of mice were observed by Snell 4). Interstrain variation in the mor- logy of freshly ovulated eggs was o noted (Braden, '59b); four inbred ins of mice differed in the degree aggregation of certain cytoplasmic nules in the eggs. Aggregation of these nules apparently took place at, or soon er, the first meiosis. In addition, fe- les of a noninbred strain produced eggs t varied considerably in the degree of nular aggregation, suggesting that the es controlling this characteristic act ing the haploid state of the egg after first meiosis.

Differences between inbred strains of ce in gametic function leading to ab- rmalities of maturation or development re reported by Fischberg and Beatty 2) and Braden ('57b). Variation in rmatozoon function in the female tract s indicated by the finding that the pro- tion of eggs in which more than one rmatozoon had penetrated the zona lucida was directly related to the strain male used, the type of female having, general, little influence (Braden, '58b). females mated with C57BL males, about % of penetrated eggs contained more n one spermatozoon as compared with -15% in females mated with CBA, A, RIII males. This apparently was not sed by the presence of large numbers spermatozoa about the eggs in the fe- les mated with C57BL males; the most sonable hypothesis was the phe- nenon was related to greater motility of 7BL spermatozoa in the female tract. e results of Edwards ('55) and Weir 3) also suggest the existence of strain erences in spermatozoon function. Ed- rds inseminated mice with mixtures taining equal numbers of spermatozoa m males of each of several inbred ins and found that the different strains not sire equal numbers of offspring. ir showed that strains of mice selected either high or low blood pH differed in sex ratio of the offspring, and that this s not attributable to selective mortality. results may, however, betoken abnor- l spermatogenesis rather than func- al variation between X- and Y-bearing rmatozoa in the female tract.

INFLUENCE OF A SINGLE LOCUS ON GAMETIC PHENOTYPE

We shall now return to discussion of the *T* locus. It is the only locus in the mouse for which there is unequivocal evidence of an influence on gametic function. Other loci for which there is evidence of an effect on spermatozoon morphology are *pink-eyed dilution* (*p*) in the mouse (Braden, '59b), and the loci controlling coat color in the rabbit (Beatty, '56). A radiation-induced mutant allele at the *p* locus affects the morphology of the spermatozoon head, though spermatozoon motility is normal (Hollander, '59).

In males that carry one *t* allele, or a *t* allele and the *T* allele, some of the spermatozoa produced have deformed heads, but the incidence is not much above normal (Bryson, '44; Braden and Gluecksohn-Waelsch, '58). In males carrying two *t* alleles (two different *t* alleles, or homozygotes in the case of viable alleles), however, the proportion of spermatozoa with deformed heads is generally much higher (range 8–48.5%). Males heterozygous for two *t* alleles, at least one of which is a homozygous lethal, are almost always very infertile, but the infertility is not correlated with the incidence of deformed spermatozoa.

The infertility of males heterozygous for two *t* alleles is a manifestation of the influence of the *T* locus on spermatozoon function. Females of similar genotypes are fully fertile. The male infertility cannot be attributed to the incidence of spermatozoa with deformed heads, to decreased motility or lifespan of spermatozoa in the female genital tract, or to interaction of spermatozoa carrying different *t* alleles (Braden and Gluecksohn-Waelsch, '58). Rather, the evidence indicates that spermatozoa from such males are incapable of traversing the uterotubal junction. Spermatozoa that do get into the tubes are apparently fully capable of fertilizing the eggs. In the mouse and rat the uterotubal junction is a very considerable barrier to spermatozoon passage. In the mated rat, the uterus contains about 60×10^6 spermatozoa compared to about 900 in the Fallopian tubes (Blandau and Odor, '49). In mice the number of spermatozoa in the uterus is of the same order as in the rat,

and the number entering the tubes is only 450–700.

Males carrying only one *t* allele are usually fertile but the transmission ratio of *t* and the wild-type allele, +, or the mutant, *T*, is abnormal (Chesley and Dunn, '36, Dunn and Gluecksohn-Schoenheimer, '39; Dunn, '57). Again, only males exhibit the phenomenon. A search for evidence of meiotic irregularities, extra divisions of spermatids bearing the *t* allele, or differential viability *in utero* of zygotes that had arisen from + — or *t*-bearing spermatozoa, proved fruitless (Bryson, '44). Instead, the evidence indicated that the *t* allele affects the function of the spermatozoon in which it finds itself in such a way that there is a difference in behavior in the female tract between + — and *t*-bearing spermatozoa (Braden, '58a). This was shown by mating T/t^0, T/t^1, T/t^{12}, and T/t^3 males with +/+ females either at the normal time (i.e., before ovulation) or several hours later. In seven of eight males tested there was a significantly lower transmission ratio in the late matings.

Following a suggestion by N. Bateman, I have been examining the transmission ratios of T/t and $+/t$ males, using females of the genotypes +/+, +/*T*, +/*t*, and *T*/*t*. I have used the alleles t^0, t^3, t^9 and t^{12}, of which all except t^3 are recessive lethals. The animals used were derived from pairs of T/t^0, T/t^3, T/t^9, and T/t^{12} animals kindly sent me by Dr. S. Gluecksohn-Waelsch. Crossing to an outbred albino strain introduced the wild-type allele, producing the genotypes +/*T* and +/*t*. Females were run continuously with males, and the time of mating was assumed to be normal. Females were segregated before parturition, and the progeny classified according to tail morphology as soon as possible after birth. The genotype +/*T* has a shortened tail, *T*/*t* has only a rudimentary tail, and t^3/t^3 and +/*t* have tails of normal length. Normal and short-tailed offspring were examined with the aid of a stereoscopic microscope.

An indication of the extent of error in genotype classification was obtained from matings for which +/+ males and +/*T* or *T*/*t* females, or +/*T* males and +/+ or +/*t* females were used, for there is a large body of evidence to show that the segregation of + and *T* in males and males, and of + and *t* or *T* and *t* in fem is normal. Two +/+ males sired a of 463 offspring from +/*T* and *T*/ males; the numbers of normal-tailed short-tailed progeny for the two type female were 98:118, and 115:132 spectively. The departures from the pected 1:1 distribution (+/+:+/*T* +/*t*:+/*T*, respectively) were not sig cant ($p < 0.2$, $p < 0.3$). In mating which +/*T* males were used, +/+ fem produced 55 normal-tailed and 40 s tailed offspring, and the +/*t* females duced 45 normal-tailed (+/+ and + 32 short-tailed and 24 tailless offsp The expected ratios for the two ma types are 1:1 and 2:1:1, respectively. results do not show significant depa from expectations ($p < 0.2$, $p < 0.3$). ther evidence on phenotypic overlap can be derived from matings involving males and +/*T* females where the expe ratio of normal-tailed (+/+ and +/*t*) spring to the combined total of short-t and tailless offspring is 1:1. Data are a able for three +/t^{12}, three +/t^3, and +/t^0 males (table 4). The over-all t obtained were 226:259, which do no part from expectation significantly (0.2). It is to be noted, however, tha four of the five sets of results given t is a deficiency of normal-tailed offsp suggesting a regular bias in the classi tion in favor of short-tailed progeny alternatively, a lower viability of +/+ +/*t* progeny.

Transmission ratio of t and T for + eggs

In view of the known tendency for m of the same genotype (with respect locus) to vary in transmission ra (Dunn and Gluecksohn-Schoenheimer, Dunn, '43), individual males were m with females of several genotypes and results from each male treated separa in the first instance.

T/t^{12} *males.* Three males were u each being mated to +/+ females f two out-bred strains, and to +/*T*, + and +/t^3 females and with one male, to +/t^9 females. The numbers of the ous types of progeny obtained are give table 1. The total number of progeny

Phenotypes are in the order normal-tailed:short-tailed:tailless

Male genotype	Male no.	Female genotype						
		+/+[a]	+/+[b]	+/T	+/t^{12}	+/t^{3}	+/t^{9}	+/t^{0}
T/t^{12}	3	90:6:0	23:0:0	23:4:32	47:9:3	64:12:5		
	4	50:12:0	50:15:0	45:9:48	68:8:5	78:33:8	34:9:10	
	17	27:8:0	9:7:0	8:9:17	24:14:8	10:14:5		
T/t^{3}	9	31:71:0	36:77:0	30:58:28	40:38:30	33:52:50		
	10		25:33:0	11:29:20	24:24:13			
	21	10:25:0	7:20:0	13:26:5	12:28:21	7:17:14		
T/t^{0}	8	52:18:0	49:14:0	7:6:5				12:6:3
	19	72:7:0	45:25:0	26:27:39		6:6:5		49:24:15
	23	65:25:0	29:14:0	46:16:41				46:13:10
T/t^{9}	13	6:15:0		9:13:10	15:27:28		13:26:6	
	18	10:40:0		4:21:8	8:20:11	20:32:27	9:22:4	
	25	9:29:0		4:9:2	1:6:1	11:15:12	5:10:7	

[a] Outbred albino stock.

[b] L.A.B. gray outbred stock.

sified for males 3, 4, and 17 was 318, 482, and 160, respectively.

Following N. Bateman ('60), I propose to use "s" to represent the male transmission ratio of t and T, or t and +, with a subscript to indicate the genotype of the eggs used. There was no significant difference between the transmission ratio, s_+, for the two strains of +/+ females, but there was appreciable heterogeneity between the ratios for the three males ($p < 0.001$). For males, 3, 4, and 17, s_+ was 0.95, 0.79, and 0.71, respectively.

The value of s_+ can also be calculated for + eggs in +/T and +/t^{12} females by comparing the proportions of normal-tailed and short-tailed progeny (see figures 1 and 2). Chi-square analysis of the proportions of these phenotypes in the progeny of +/+ (albino), +/+ (L.A.B. gray), +/T, and +/t^{12} females for each male showed no significant heterogeneity ($p < 0.2$, $p < 0.5$, and $p < 0.2$, respectively).

T/t^{3} males. Three males were mated with +/+, +/T, +/t^{12}, and +/t^{3} females (table 1). The total number of progeny classified for males 9, 10, and 21, was 574, 179, and 205, respectively. There was no significant heterogeneity between males or between female genotypes +/+ and +/T in the proportions of normal-tailed and short-tailed progeny. Because t^{3}/t^{3} is viable s_+ cannot be readily determined in +/t^{3}

Fig. 1 The progeny genotypes and phenotypes from T/t^{12}♂ × +/T♀ matings, and results from three T/t^{12} males.

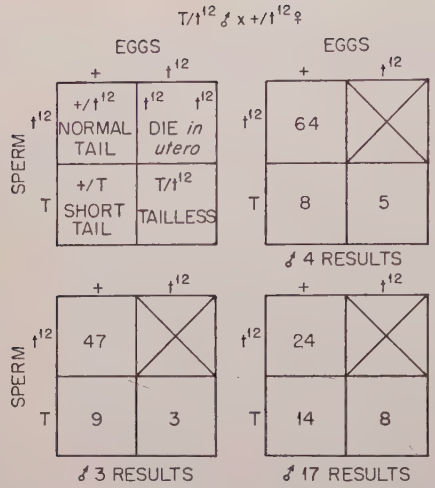


Fig. 2 The progeny genotypes and phenotypes from T/t^{12}♂ × $+/t^{12}$♀ matings, and results from three males.

females. Combining data from +/+ and +/*T* females, s_+ for males 9, 10, and 21 was 0.32, 0.37, and 0.30, respectively.

T/t^0 males. Data are available from three males (table 1). Analysis of the proportions of normal and short-tailed offspring from +/+, +/*T*, *and* $+/t^0$ females revealed that, whereas there was no appreciable heterogeneity in the data from males 8 and 23 ($p < 0.5$ and $p < 0.7$, respectively), the results from male 19 showed highly significant heterogeneity ($p < 0.001$). This was largely attributable to a very high s_+ for the albino strain of +/+ females (0.91, as compared with 0.64 for L.A.B. gray, 0.49 for +/*T*, and 0.67 for $+/t^0$ females). When this group was excluded, the remaining heterogeneity in the groups from male 19 was not significant ($p = 0.1$). The cause of the aberrant ratio is obscure.

T/t^9 males. In spite of outcrossing to the albino outbred stock, males carrying t^9 were rather infertile, and as a result, the numbers of progeny were not great. Three males were used (table 1). Again there was no significant heterogeneity between males, or between female genotypes in the proportions of normal- and short-tailed offspring. For males 13, 18, and 25, s_+ was 0.34, 0.22, and 0.30, respectively.

Transmission ratios of t and T for T eggs

Figure 1 shows that s_T for T/t males c only be obtained indirectly. Two assum tions must be made: (1) +/*T* femal produce equal numbers of + and *T* eg and (2) *T*/*t* and +/*t* embryos are equa viable. The evidence given as well as th of other workers (Dunn, '39; Dunn a Gluecksohn-Schoenheimer, '39; Smith, '5 Bateman, '60) indicates that the assum tions are reasonable. Then if $s_+ = s_T$, t number of *T*/*t* progeny should be equal the number of +/*t* progeny in T/t♂ +/*T*♀ matings. Data from matings of t type (detailed in table 1) are summariz in table 2. The results do not differ signi cantly from expectation ($p = 0.2$), that s_T is similar to s_+. The data are similar those of Dunn and Gluecksohn-Waels ('53) for T/t^3, T/t^9, and T/t^{12} males (table 2) and of Dunn and Glueckso Schoenheimer ('39) for T/t^1 males (3 normal-tailed, 325 tailless progeny).

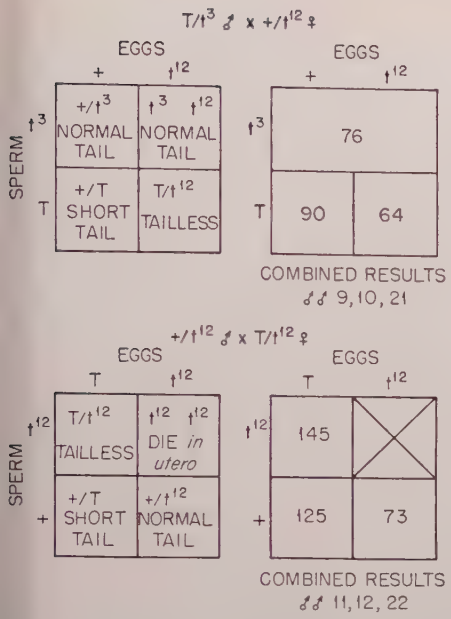
TABLE 2

The relative numbers of normal-tailed an tailless progeny from T/t ♂ × +T/♀ matin

Male genotype	No. of males	Number of proge	
		Normal-tailed	Tail
T/t^{12}	3	76	9
T/t^3	3	54	5
T/t^0	3	79	8
T/t^9	3	17	2
Total	12	226	25
Data of Dunn and Gluecksohn-Waelsch ('53			
T/t^{12}	—	32	3
T/t^3	—	79	10
T/t^9	—	73	6
Total		184	19

Transmission ratios of t and T for t eggs

As in the case of s_T, the transmiss ratio s_t cannot be obtained directly a similar assumptions must be made. matings of the type T/t♂ × +/t♀, the nu bers of +/*T* and *T*/*t* progeny should similar if $s_+ = s_t$ (see figs. 2 and 3). Fr the combined results of matings of t type (table 3), it is obvious that s_t diff from s_+. In an earlier section it was sta that there appeared to be a classificat bias in favor of short-tailed progeny.



'ig. 3 The progeny genotypes and phenotypes n $T/t^3♂ \times +/t^{12}♀$, and $+/t^{12}♂ \times T/t^{12}♀$ mat-s and results obtained.

ıtrol matings from which $+/t$ and $+/T$ spring were expected in equal numbers, observed numbers were 115 normal l 132 short-tailed, a ratio of 0.466: 34. In matings from which normal-ed ($+/+$ and $+/t$) progeny and short-ed and tailless progeny were expected he ratio 0.5:0.5, the observed ratio was 5:259 or 0.466:0.534. A χ^2 contingency was performed with the experimental a (table 3) and the combined control a just cited ($\chi^2_{(1)} = 8.26$, $P < 0.005$). nore direct control is the mating $+/T♂$ $-/t♀$, but the number of progeny ob-ıed so far is small (45 normal-tailed, 32 rt-tailed, and 24 tailless). Published data of other workers in which the mating $+/T♂ \times +/t♀$ was used indicate only a slight excess of short-tailed over tailless progeny ($+/t^1$ females, 312:319, Dunn and Gluecksohn-Schoenheimer, '39; $+/t^{12}$ females, 1095:1128, Smith, '56). The results reported in the preceding section support the conclusion, for there was in those matings an excess of tailless over normal-tailed progeny (table 2). It seems, then, that the proportion of eggs fertilized by *t*-bearing spermatozoa is higher for eggs that have a *t* allele than for those with a + allele, and this within the same female.

TABLE 3

The relative numbers of short-tailed and tailless progeny from $T/t♂ \times +/t♀$ matings

Parental genotype		Number of progeny	
Male	Female	Short-tailed	Tailless
T/t^{12}	$+/t^{12}$	31	16
T/t^3	$+/t^{12}$	90	64
T/t^9	$+/t^{12}$	53	40
Total		174	120
T/t^{12}	$+/t^3$	59	18
T/t^3	$+/t^3$	69	64
T/t^9	$+/t^3$	47	39
Total		175	121
T/t^{12}	$+/t^9$	9	10
T/t^9	$+/t^9$	58	17
Total		67	27
T/t^0	$+/t^0$	43	28
Grand total		459	296

Transmission ratios of + and t for T and t eggs

$+/t^{12}$ *males.* The number of progeny sired by three $+/t^{12}$ males from $+/T$, T/t^3 and T/t^{12} females is given in table 4. The transmission ratio s_T can be calculated

TABLE 4

Numbers of progeny according to phenotype from various mating classes (+/t males)
Phenotypes are in the order normal-tailed:short-tailed:tailless

Male genotype	Male no.	Female genotype			
		+/T	T/t^{12}	T/t^3	T/t^9
$+/t^{12}$	11	77:49:39	34:51:26	52:32:19	
	12	38:14:21	15:28:43	27:17:20	
	22	27:17:19	24:46:76	31:38:29	
$+/t^3$	29	22:11:6	25:30:9	1:7:6	6:13:5
	30	3:4:2	15:17:3	0:2:1	
	31	27:27:6	13:8:3		
$+/t^0$	38	32:21:23			

from the numbers of tailless and short-tailed progeny (fig. 3). For males 11 and 12 there was no significant heterogeneity in the results from the three female genotypes, but there was for male 22($P < 0.05$). Combining female genotypes, s_T for males 11 and 12 was 0.39 and 0.59, respectively.

From the progeny of the mating $+/t^{12}\delta \times T/t^{12}\varphi$, an estimate of s_t can be obtained indirectly. If the numbers of T and t^{12} eggs produced are similar, and $+/T$ and $+/t^{12}$ offspring are equally viable, the ratio of short-tailed and normal-tailed offspring would be approximately 1:1 when $s_t = s_T$. The results show a significant excess ($P < 0.05$) of short-tailed progeny (125 short-, 73 normal-tailed). Allowance for the bias in favor of short-tailed progeny was again made in the analysis.

DISCUSSION

The results just described indicate that, in general, the paternal transmission ratio of t and T for $+$ eggs (s_+) does not vary significantly with the maternal genotype. They also show, as do the findings of Dunn and Gluecksohn-Schoenheimer ('39) and Dunn and Gluecksohn-Waelsch ('53) that, at least for T/t^0, T/t^3, T/t^9, and T/t^{12} males, the transmission ratio for T eggs (s_T) is apparently similar to s_+. The findings of Bateman ('60), on the other hand, indicate a significant ($p = 0.005$) difference between s_+ and s_T for T/t^e males. Bateman has also found that for $+/t^e$ males s_t is less than s_T ($p = 0.013$). In the present investigation using t^0, t^3, t^9, and t^{12}, s_t calculated for T/t males was significantly higher than s_+; likewise, for $+/t^{12}$ males $s_t > s_T$. In contrast, the paternal transmission (or segregation) ratios of $+$ and T are normal (0.5) for both T and t eggs (Dunn, '39; Dunn and Gluecksohn-Waelsch, '53; and present results). It is to be noted, however, that s_T and s_t for T/t males and s_t for $+/t$ males can be estimated only indirectly, and the conclusions just stated depend on certain assumptions. Evidence from various control matings suggest that these are reasonable.

As already mentioned, Bateman using only the allele t^e found that $s_+ > s_T > s_t$, whereas in the present work with t^0, t^3, t^9, and t^{12} it appears that $s_t > s_+ = s_T$. This suggests that the different t alleles vary in the direction and magnitude of their e on spermatozoon–egg interaction and cordingly, we find significant heteroge ($p < 0.005$) between (but not within) various mating types (table 3) in the portion of short-tailed and tailless spring.

Two types of mechanism may be visaged for the apparent variation in ternal transmission ratio of t and T cording to egg genotype. It is of cri importance in this connection that only first meiosis has been completed at time of spermatozoon penetration. I duction at the T locus usually occurs a second meiotic division, most of the ovulated in females heterozygous at locus will have identical genetic cons tion until after spermatozoon penetra is complete. In other words, there i possibility of selective attraction betw eggs and spermatozoa carrying spe alleles at the T locus. Nevertheless, e of a spermatozoon carrying a t allele m affect the egg in such a way as to c preferential retention of the egg's t a and expulsion of the complementary a in the second polar body. Such a me nism would result in aberrant mate segregation ratios of $+$ and t or T a These are not, in fact, found. We are therefore, with the alternative that reduction occurs at the T locus, resu in eggs of two types being ovulated i males heterozygous at this locus, and, thermore, that eggs carrying a spe allele are preferentially penetrated b bearing spermatozoa.

These considerations raise an issu the field of the physiology of fertiliza for they imply something akin to ch taxis, a phenomenon that has not yet demonstrated in animals (Tyler '55) animals where fertilization is normall ternal, it may well be that chemotaxi erates but that we have not had the niques necessary for its demonstra Perhaps genetics has now provided us such a tool in the mouse. In a recent s of the spatial distribution of spermat about the eggs in mice, it was pointe that chemotaxis was a possible exp tion of the findings (Braden, '59a).

To return to the main theme—sele fertilization requires that the alleles a

rticular locus find expression in the gam- phenotype and, further, that gene ac- is postreductional. Gene action is dently postreductional in T/t and $+/t$ les and females, but prereductional as ards spermatozoon function and mor- logy in males heterozygous for two t eles. Two other observations that have en mentioned are suggestive of postre- ctional gene action. They are the aggre- ion of cytoplasmic granules in mouse gs (Braden, '59b) and the melanizing ac- ity of rabbit spermatozoa in the pres- ce of dihydroxyphenylalanine (Beatty,). In human ejaculates, Gullbring ('57) s demonstrated by serological methods segregation of the blood group anti- ns A and B. Spermatozoa were either of or B type. We may conclude that the dence shows that genes can express emselves in the phenotype of the gamete d, with one locus at least, the action is streductional and affects function in ch a way as to cause marked departure m the Mendelian postulate of random ion of the gametes.

OPEN DISCUSSION

AUSTIN[1]: You mentioned that the rate cumulus maturation seems to differ h the genotype of the female. Is this by ect observation or by inference?

BRADEN: This is by inference. The time ovulation as far as the evidence went s the same in C57BL and CBA females, the time of sperm penetration was lier in C57BL females than in CBA fe- les. It seemed to be related to density the cumulus, but the assessment was jective.

AUSTIN: I thought you did observe what peared to be a denser cumulus in some the animals.

BRADEN: In CBA as compared with 7BL females. This, however, was several rs after ovulation and may not reflect initial difference in density, but rather nore rapid dissolution.

AUSTIN: Differences in cumulus den- would presumably affect its perme- lity—could not your results be ex- ined on this basis, rather than by in- ing chemotaxis?

BRADEN: It is unlikely because the re- ts indicate differences between eggs from the same female. It would involve an effect by the gene on cumulus density after the first meiosis.

LINDSLEY[2]: The transmission ratio of T- versus t-bearing sperm from a T/t heterozygote shifts in favor of the t sperm with increasing interval between insemination and fertilization, as demonstrated by your delayed mating results. This would seem to indicate that the fertilizing ability of T-bearing sperm is decreasing with time, even though sperm of identical genotype from T/t males display constant functional ability. An alternative explanation of the delayed mating results, however, might be that t-bearing sperm are becoming more functional with time. Have you any way of distinguishing between these alternatives?

BRADEN: If the fertilizing ability of the T-bearing sperm were decreasing with time, we would expect an effect of late mating on the segregation ratio of $+/T$ males. As far as I know, this has not been investigated.

WAELSCH[3]: Could not some of your data be explained by differential viability of certain tailless embryos compared with short-tailed embryos? I notice you have a particularly high excess of short-tailed in matings of T/t^9 males by $+/t^9$ females; whereas similar matings with T/t^{12} males and $+/t^{12}$ females do not give such an excess. Have you considered the possibility of differential viability?

BRADEN: Yes. In $T/t♂ \times +/T♀$ matings (table 2), there was a slight excess of tailless over normal-tailed offspring; and in the control matings, there was a slight excess of short-tailed over normal-tailed offspring. There is therefore no evidence of lower viability of tailless embryos. Variation between different t alleles in their effect on the egg is likely. Bateman's work shows that the effect of t^e is in the opposite direction to that of t^0, t^3, t^9, and t^{12}.

WAELSCH: Is there a possibility, particularly in the case of t^{12}, that an effect takes place on the eggs themselves in the heterozygote similar perhaps to the effects of t^{12}

[1] C. R. Austin, National Institute for Medical Research, London.

[2] D. L. Lindsley, Oak Ridge National Laboratory.

[3] Salome G. Waelsch, Albert Einstein College of Medicine.

or other *t* alleles on the sperm? Pamela Deakin, in our laboratory, has prepared some preliminary data in the case of t^4 where it looks as though you might get deviation from the expected 1:1 ratio in males in the opposite direction from that in females; whereas $+/t^4$ males show an excess of + gametes $+/t^4$ females seem to show a deficiency of the *t* allele. I think that your data would encourage the belief that some *t* alleles have an effect on the eggs as well as on the sperm.

BRADEN: That is my thesis—that the *t* alleles affect the function of the egg at or about the time of sperm penetration. But I think the control matings (both mine and others) imply that the segregation ratio of + and *t*, or *T* and *t*, in females is relatively normal, except for t^4 perhaps.

CHAIRMAN DUNN[4]: One of the main points made by Dr. Braden appears to me to assume that *t* genes act to produce differential behavior of the spermatozoa. The difference in number of zygotes at birth (95% or in the case of t^{12}, 80-odd %) referred to physiological differences in the two kinds of sperm after reduction. Does the assumption here have any bearing on the differential behavior of eggs, or are these two separate phenomena? The demonstration that there may be differences in the action on the egg does not affect your main conclusion, I take it, that the main evidence of action of genes in the sperm in mammals is based on the male transmission ratio. Perhaps Dr. Waelsch's remarks indicate that another comment on the relationship of these two phenomena that you have been describing would be appropriate.

BRADEN: The data on late mating of T/t males with $+/+$ females indicate that *t* genes affect sperm function and at least part of their action comes into expression after the sperm is introduced into the female genital tract. Then the data that I have presented today, and Dr. N. Bateman has similar data, indicate that *t* genes seem to affect the function of the egg as well.

OWEN[5]: Males heterozygous for translocations produce sperm that carry duplications and deficiencies. These sperm function at fertilization and the embryos with unbalanced constitutions die. I wonder if this does not suggest that the phy ology of the sperm is not generally s sitive to its genic constitution.

BRADEN: That is likely. The evide we have got from "good" genes indica that they do not affect sperm funct (otherwise they would not be "go genes). However, the *t* alleles would pr ably not have been found if the *T* all was not available as a "tester." It is j possible that there are other genes in mouse or other species that have not b detected because no suitable tester all has been found.

L. B. RUSSELL[6]: The data on 'dse' ficiencies, which I shall present later the Conference, suggest that the *t* lo may not be unique in affecting the fu tion of gametes. There is at least a possi ity that the transmission of certain 'd deficiencies is reduced.

LINDSLEY: I think that a remark ab the interaction of paternal and gam genotypes in the ability of *Drosoph* sperm to function in fertilization might pertinent to the interesting results on *t* locus just presented. After an appro ate meiotic event, it is possible to obt spermatids deficient for a sex chromoso a second, a third, or a fourth chomoso These grossly deficient spermatids are pable of developing into functional spe Yet from certain paternal genotypes obtain results that indicate differen functioning in fertilization by recipro products of meiosis. So, in *Drosophila* we have the paradox that differen functioning of gametes of different ge types occurs, although in most situati gametic function appears to be indepe ent of gametic genotype.

BRADEN: Do you imply that gene act in that case is prereductional?

LINDSLEY: Yes, it is the genotype of spermatocyte that is important, and t somehow determines which sperm ge types are going to function. It is conc able that the *t* determines what happ to the *T* segregant somehow. The

[4] L. C. Dunn, Columbia University.

[5] R. D. Owen, California Institute of T nology.

[6] Liane Brauch Russell, Oak Ridge Nati Laboratory.

t the *T* has been in the same spermato-
e with the *t* is what is important.

VILKIE[7]: I do not know how favorably
would regard the spermatozoa of
chen; probably you would regard the
vements in a favorable light. Some
rs ago I discovered that the sperm carry
ompatibility genes and worked out their
de of inheritance. This indeed seems to
a case of the gene producing its effect in
sperm.

BRADEN: There is a reasonable amount
evidence to indicate that genes may
ction in the gametes in plants.

VILKIE: This is the only case of its
d with motile sperm.

BRADEN: Yes. I think it is only in
ns, mosses, and seaweeds that there is
d evidence for chemotaxis.

VILKIE: Again I think it was only in
chen that chemotaxis was proved to
e place.

KAPLAN[8]: Some years ago I studied the
cess of fertilization in ferns, and the
ervations made at that time indicated
t chemotaxis probably does not operate.
at seems to draw the sperm into the
hegonium is a sudden bursting of the
hegonium, the contents of which are
cted, creating a slight vacuum. Sperm
mming about in the surrounding water
carried into the archegonium in the
er that rushes in to replace the arche-
ial contents.

STERN[9]: I would like to make a remark
arding, not abnormal segregations, but
mal ones.

You stated correctly that it has usually
n assumed that the genes are inactive
the male postmeiotic period. This as-
mption is based on the fact, first stressed
Muller, that sperm carrying deficiencies
ethals appear to have normal viability.
re is some recent electronmicroscopic
dence that may affect this assumption.
ycett and Ito have shown the regular
stence of intercellular bridges connect-
spermatocytes and spermatids. This
kes the four spermatids actually part of
cytial unit. Therefore, two spermatids
, as a result of segregation, lack cer-
genes may be cross-fed by the two
rmatids in which these genes are pres-

The existence of normal segregation
os may thus depend on genic action in the early spermatid nuclei. Abnormal segregation ratios may sometimes occur when the genic products do not move speedily or efficiently across the intercellular bridges.

It may be desirable to distinguish phases of genic action not only as pre- and postmeiotic but to subdivide the postmeiotic phase into two parts—the early spermatid stage when the nuclei are large and possibly genetically active and the sperm stage with its condensed nuclei and a possibly inactive genic content.

BRADEN: That is a good point. The gene action comes into evidence in the fully formed sperm in the female tract; but the initial action may well have occurred in the stage where the four spermatids were interconnecting. But the situation is quite different in the egg. The first meiosis is completed only just before ovulation and a few hours before fertilization, and the second is not completed until after the sperm is penetrated.

WAELSCH: I was worried also about the differentiation between the prereductional and postreductional effects, particularly in respect to the compound males that are sterile. It is hard for me to visualize that these alleles would have a prereductional effect when present together in compounds and a postreductional effect when present in heterozygotes. Possibly Dr. Stern's comment on Fawcett's work might provide a hypothesis. I have given a lot of thought to this problem and formulated some hypotheses. I do feel that it would somehow be more satisfactory to tie up the effect of *t* in the heterozygous condition with the effect of *t*'s when present together, and at least perhaps refer them to the same phase —either pre- or postreduction.

BRADEN: I think that is right, because the *t* alleles have a number of effects apart from that on segregation ratio.

BATEMAN[10]: On the face of the question of the genotype of the egg affecting the selection of the sperm, it seems very difficult to see how it would operate, for as you just said the first division takes place very shortly before fertilization and the cytoplasm of the egg has been formed con-

[7] David Wilkie, University of Washington.
[8] W. D. Kaplan, City of Hope Hospital.
[9] Curt Stern, University of California, Berkeley.
[10] A. J. Bateman, Christie Hospital, Manchester.

siderably before. But there does seem to be another possibility—that it is not the genotype of the egg that is determining the selection of the sperm, but the genotype of the polar body, where the nucleus represents practically the whole of the polar body, which in many cases disintegrates very early into the perivitelline space. So the space around the egg may well contain material from the polar body, which may be actually determining the selection of the sperm. There is a complete negative correlation, of course, between the polar body and the egg. It may seem at first sight just an academic point, but it may be the genotype of the polar body that is determining the selection rather than that of the egg.

BRADEN: Yes, I think that is quite possible.

OWEN: Your conclusions from Gullbring's observations on the sperm of AB men were drawn with more confidence, I believe, than many of us would feel justified at present. It is not enough to show that sperm populations can be subdivided in mixed agglutination systems; it is necessary to test whether the free sperm remaining do indeed transmit the allele that they are postulated to carry.

Dr. D. Schmidt of Munich and Dr. W. H. Stone of the University of Wisconsin have permitted me to cite a manuscript reporting an intensive search for effects of red cell antigen-controlling loci on the sperm of cattle, where there is a good deal of diversity and many loci are marked. Except for characteristic J, these authors find no evidence of any red cell antigenic specificities on the sperm surface or segregating in the sperm of segregating males. J is an antigen found in the blood and seminal plasmas that is secondarily adsorbed to red cells and seems also to be secondarily adsorbed to sperm. So, even in the heterozygous male, all of the sperm presumably display the J specificity.

I submit this only because it seems to me there is as yet no good reason to expect that a sperm cell will express its own haploid genotype for cellular antigens controlled by genes segregating in the sperm population.

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mbryological Phases of Mammalian Gametogenesis[1]

BEATRICE MINTZ[2]
Whitman Laboratory of Experimental Zoology, The University of Chicago, Chicago, Illinois

The origin and history of mammalian metes has remained obscure and con-versial despite the critical place that ese cells occupy in heredity and ontog-y. There has in fact been some ques-n as to when, in the chronology of an dividual, the study of oogenesis and ermatogenesis becomes relevant since, cording to some views, any germ cells at may form during prenatal life do not ntribute directly to the definitive re-oductive line.

The observations to be summarized here e derived chiefly from studies of the ouse, a species in which special genetic d histochemical features have made the abryonic phases of gametogenesis more cessible to analysis. These investiga-ns establish that the germ cells make eir appearance early in embryonic life, d that mitotic descendants of the same l lineage persist to maturity in the adult, both males and females. Prenatal ents in gamete development are, more-er, numerous and complex. They are dispensable antecedents to postnatal anges and their course may be influ-ced from the very beginning by both vironmental and genetic factors, with ofound long-range consequences.

1. EARLY PRIMORDIAL GERM CELL DEVELOPMENT IN NORMAL EMBRYOS

Although germ cells may be very num-ous in the adult mammal, particularly the male, all the definitive ones are rived from less than 100 first seen in e endodermal yolk sac epithelium local-ed near the base of the allantois. This age in the mouse is at 8 days of develop-ent, when the embryo has only a few irs of somites. The smallest germ cell mber thus far seen in any 8-day individ-l is 10; these might, in turn, have fewer ecursors. In the mouse, as in a few other species, the cytoplasm of the primordial germ cells is rich in alkaline phosphatase. This precocious circumstance of chemo-differentiation renders them unusually con-spicuous in histochemical preparations and readily distinguishable from all other cells at that time (Chiquoine, '54; Mintz and Russell, '55, '57). Subsequently they enter the gut. At 9–12 days, as the paired germinal ridges make their appearance, the germ cells migrate—apparently ac-tively and probably selectively—up the dorsal gut mesentery and into these ridges. Corresponding cells appear in the yolk sac of the human embryo, where they are unusually large, relative to somatic cells (Witschi, '48) and these also have been observed in migration to be phosphatase positive (McKay *et al.*, '53). A quantita-tive census of the mouse germ cells, made possible through the ease with which all of them may be identified, reveals that the original few have multiplied to 5000 or more when the migratory period termin-ates (Mintz and Russell, '57). Mitotic figures are visible if nuclear staining is added, and they indicate no synchroniza-tion of divisions.

Even before the end of migration, or of histogenesis of somatic cells in the gonad primordia, the earliest sex difference ap-pears. At 11 days of embryonic life, a dif-ferential deployment is evident among those germ cells that have reached the ridges, such that the distribution is dis-tinctly either peripheral (fig. 3) or central (fig. 4). The former is associated with pro-gressive cortical development, and is there-fore found in the female; the latter is fol-lowed by increasing medullary dominance,

[1] These studies have been aided by P.H.S. re-search grant C-3502CB from the National Cancer Institute, Public Health Service.

[2] Present address: The Institute for Cancer Research, 7701 Burholme Avenue, Philadelphia 11, Pa.

in the male. The epithelium of the ridge in the male concurrently develops a slightly phosphatase-positive reaction. This initial phase of primary sex differentiation is exceedingly rapid, occupying less than 1 day in embryonic life. Although the definitive somatic organization of the gonad is realized more slowly, the short duration of what may be the most labile period may be a contributing reason for the rarity of intersexuality in the mouse. Despite the large number of animals that must come under surveillance, only the one case reported by Fekete and Newman ('44) involves an ovotestis likely to have arisen by reversal of the genetically determined balance between cortex and medulla. (Gynandromorphs such as described by Hollander *et al.*, '56, probably are of different origin.) The comparable transitional period in human ontogeny requires a longer time (Witschi, '56).

Further development of germ cells in female and male will be taken up in Sections III and IV, respectively.

II. GENETIC CONTROL OF EARLY GAMETOGENESIS

Effects of mutations at certain single loci

The increase in primordial germ cell number that normally takes place during migration fails to occur in the mouse if the embryo is homozygous for mutant genes at either the *W* or the *Sl* locus.

Mutant alleles that may be substituted for the wild-type *w* are *W*, W^v, or W^j, and the homozygotes or compounds of these are largely or totally deficient in germ cells (fig. 5, 6). Substitution of the mutant *Sl* allele for both *sl* genes similarly renders the homozygote defective.

Surviving homozygous mutant males and females of the *W* series are sterile in adult life. Fertility is unimpaired in heterozygotes, and matings of these yield 25% of the deficient type. The presumptive steriles are detectable in the expected proportions as early as the 9th day of embryonic life, on the basis of total counts of the phosphatase-positive cells located between yolk sac and gonad primordia, and it is this statistical correlation that has served to establish identity of the cells in question. Compared with normal controls and with the remaining 75% of their o littermates, the mutants continue to sh a low number of germ cells (Mintz a Russell, '55, '57; Mintz, '57a). Althou the primordial germ cells form, they sh little or no capacity for the mitotic activ that normally characterizes them dur this period. It is the persistence of t defect, first expressed in the embryo, t causes sterility at reproductive age. Ea observations of embryos from heterozyg *Sl/sl* matings have confirmed the ex ence of a comparable adverse influe of the *Sl/Sl* genotype (Bennett, '56).

The mutant genes of the *W* and series share the property that they pleiotropic, and it is striking that they p duce the same kind of syndrome involv blood, pigment, and germ cells and leadi respectively, to anemia, absence of c color, and sterility in homozygotes. It been pointed out in an earlier discussion the *W* locus syndrome that the germ defect does not occur simply as a con quence of the anemia in the same anim as it is already present before the ane is diagnosed (12½ days) and before embryonic circulation is established su ciently as to make such an anemia ph iologically critical, even if it were actua initiated during yolk sac hematopoie The abnormality may stem from a sin gene-mediated change to which these p ticular cell types are peculiarly vuln able, owing to some strategic common quirements in their early developm (Mintz and Russell, '57; Mintz, '57a). would be expected, removal of defect *W/W* or W^v/W^v fetal gonads to an proved nonanemic environment such normal adult spleen (Russell et al., '5 or explantation of 12-day *W/W* gonads a favorable organ culture medium just fore appearance of anemia (Borghese, '5 does not alleviate the germ cell defe which is already fully expressed at 9 da

In the case of *flexed*, there is a tra tory anemia of the intermediate hema poietic generation in the fetus (Grüneb '42); it has no effect on fertility in adult. Mice with the genotype *an/an* anemic as well as sterile. Although ma germ cells are present at birth in b sexes, they degenerate thereafter and, e if transfusions are administered to p

ıg the life of the animal, no germinal provement occurs (Menner, '57).

Interaction of nonallelic genes

The effects on embryonic gametogenesis combining mutant genes at the indendently segregating *W* and *Sl* loci have en examined in a series of 243 embryos ɔm 9 types of matings (table 1), segregatg different genotypic ratios. A preliminy report of the data has appeared (Mintz, 7b). In each case where the allele *W* ıs employed (matings 1, 2, 4, 5, 6, 7), e W^v allele was used alternatively in a rallel type of mating. Numbers of emyos observed at 9 days and at 10 days e shown in table 1. The genetic backound of these mice was (C3H × C57BL/ F_1 or a mixed background for *w/w Sl/sl*, */w Sl/sl*, and W^v*/w Sl/sl*; C57BL/6 for */w sl/sl* and W^v*/w sl/sl*; and WB for */w sl/sl*. Total germ cell number per ıbryo was scored, as in earlier studies Mintz and Russell, '57; Mintz, '57a), in rial sections prepared with the azo dye chnique, fast red TRN being used for upling to visualize alkaline phosphatase.[3] Results revealed the presence of only ro phenotypic classes, according to germ ll numbers. No embryo in this sample ıd a germ cell total falling between 90 ıd 120. When counts for each mating ere linearly arranged on a logarithmic ale, because of repeated doubling of cell ımber at mitoses, the span between 90 ıd 120 was the longest "blank" zone consistently found in all matings. The location of this gap is at the upper limit of germ cell numbers in normal embryos at 8 days; it therefore seems, at 9 and 10 days, to mark off a group in which no further mitotic increase has taken place.

All-normal *w/w sl/sl* controls (mating 9) yielded a minimum count of 132 at 9 days and a minimum of 291 at 10 days. Ten-day counts in matings 6, 7, and 8 fell close to the 291–794 range of *w/w sl/sl* embryos in mating 9; the former three, combined, contained between 240 and 1612 germ cells each. Since matings 6, 7, and 8 should each segregate 50% single heterozygotes (either *W/w sl/sl*, W^v*/w sl/sl*, or *w/w Sl/sl*) as well as 25% (mating 6) or 50% (matings 7 and 8) of the *w/w sl/sl* type entirely lacking in mutant genes, we can conclude, from the continuous distribution of the counts, that there is no apparent influence of any of these genes on primordial germ cell number if present in a single dose only. Examination of results from 7 and 8 also indicates no difference in effect of one *Sl* substitution as against one *W* (or W^v) in the otherwise wild-type constitution.

In mating 6, which should also yield 25% double heterozygotes, there is no

[3] Special thanks are due Dr. Elizabeth S. Russell of the R. B. Jackson Memorial Laboratory for supplying the adult animals used, as well as for sending preserved embryos from some matings. I am also indebted to Dr. Ann N. Bond for invaluable assistance in making cell counts in the study of interactions between *W* and *Sl* loci.

TABLE 1

Summary of matings in study of interaction between W and Sl loci

	Mating No.	Mating Parents	No. of embryos at 9 days	No. of embryos at 10 days	Theoretical ratio of defectives: normals
Group I	1	*W/w Sl/sl* × *W/w Sl/sl*	10+(7)[a]	15+(14)	43% : 57%
Group II	2	*W/w sl/sl* × *W/w sl/sl*	13+(9)	17+(10)	25% : 75%
	3	*w/w Sl/sl* × *w/w Sl/sl*	8	21	
	4	*W/w Sl/sl* × *W/w sl/sl*	3+(9)	8+(15)	
	5	*W/w Sl/sl* × *w/w Sl/sl*		7+(12)	
Group III	6	*W/w Sl/sl* × *w/w sl/sl*		10+(16)	0 : 100%
	7	*W/w sl/sl* × *w/w sl/sl*		5+(7)	
	8	*w/w Sl/sl* × *w/w sl/sl*		7	
	9	*w/w sl/sl* × *w/w sl/sl*	9	11	
	Total no. of embryos →		43+(25)=68	101+(74)=175	

[a] Numbers in parentheses indicate embryos from the parallel mating involving the W^v rather than the *W* allele.

evidence that a simultaneous substitution of one mutant gene at each of the two loci creates any special difficulty in germ cell proliferation or viability. The double as well as the single heterozygotes are of course fertile, but germ cell number in such adults in not known and would be far more difficult to determine. In summary, the matings of group III all yield a single phenotype—the normal one.

Groups I and II, however, consist of matings that would all be expected to have some mutant offspring. All did produce some embryos whose total germ cell number was below 90. Out of a total of 50 such mutants in groups I and II, 13 had fewer than 10 germ cells at 9 or 10 days of age, 2 mutants had only 2 cells each at 10 days of age, and 3, also at 10 days, had no germ cells whatsoever, although the embryos appeared otherwise normal morphologically. No control or mutant-producing matings have previously been seen with complete absence of germ cells at so early an age. Possibly this represents a seco ary reduction resulting from limited via ity of these cells in the mutants (Mi '57b).

The ratios obtained in groups I and are consistent with the hypothesis t genes at the two loci behave as com mentary factors. Matings between dou heterozygotes (group I) should, on t hypothesis, give a 43% :57% (7:9) ra of mutants: normals. Actual ratios w 41% :59% at 9 days (fig. 1) and 52 48% at 10 days (fig. 2). In group II, matings should yield 25% mutants: 7 normals, if complementary factors are volved. The combined 9-day matings group II showed 24% defectives:76% n mals; at 10 days, 20% :80%. *P* values all experimental ratios are shown in ures 1 and 2. The sole marked deviat from expectation in any single mat occurred in mating 2 at 10 days, wh included no mutants; the lower *P* va

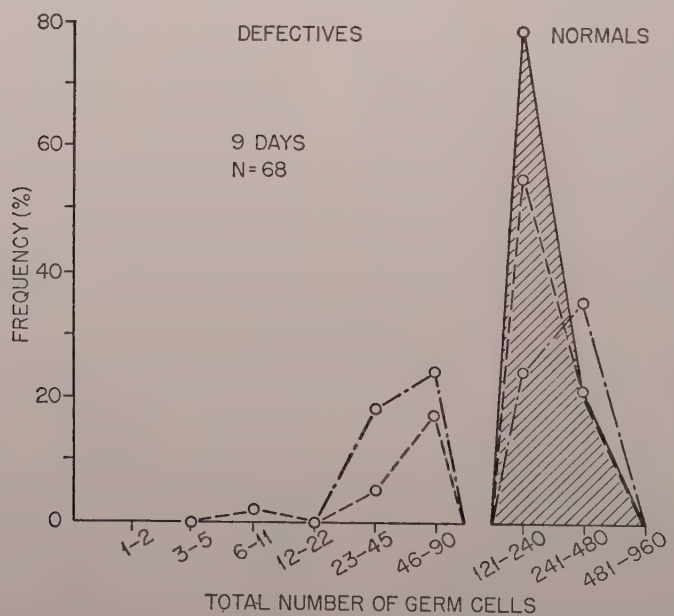

Fig. 1 Distribution of germ cell numbers in 9-day embryos segregating for genes at *W* and *Sl* loci.

		Defectives : Normals	χ^2	*P*
- — · —	Group I	41% :59%	0.04	0.80–0.90
— — — —	Group II	24% :76%	0.03	0.80–0.90
———	Group III	0% :100%		

combined matings of group II at 10
s is attributable to this deviation.
Results of all counts are summarized in
curves of figures 1 and 2. Percentage
quency of ascending ranges of germ cell
mbers is shown for each group of mat-
s. The curves are brought down to the
o frequency in the gap between 90 and
) cells, since this appears to separate
two kinds of phenotypes. The shaded
a in each figure indicates the control
ults (group III), which comprise only
mal embryos.
As long as each of the two loci is inde-
ndently but simultaneously represented
at least one wild-type gene, the normal
enotype is realized. Neither *w* nor *sl*
ne can mediate normal primordial germ
l proliferation, but both are required.
is suggests that each makes a unique
ntribution to the growth and multiplica-
n of primordial germ cells. Comple-
ntary factors have been thought to be
olved in some way in a reaction se-
ence, and it would appear possible that
W sl/sl and *w/w Sl/Sl* are defective at
ferent points in such a sequence, each
lispensable for the production of a fur-
er developmental stage. The absence of any biochemical information on reactions or syntheses special to this cell type make it impossible to speculate further on the problem.

Sarvella and Russell ('56), who first described the *Sl* mutation, point out that the *Sl/sl* heterozygote, like *W/w* and W^v/w, shows semidominant expression of the mutant gene with regard to coat pigmentation. There is some white spotting and also slight over-all dilution, as with W^v/w. The double heterozygote W^v/w *Sl/sl* is more extensively spotted and has greater pigment dilution than either component single heterozygote alone. They note that this effect, which is more than additive, suggests that the two genes affect similar processes.

Effects of genetic modifiers

The expression of specific genes is not likely to be autonomous and may be modified by factors occurring in the rest of the genome. There is evidence that this may also be true of the influence of W^v/W^v on gametogenesis.

In the embryological studies of germ cells in W^v/W^v mice (Mintz and Russell, '55, '57), the animals used were isogenic

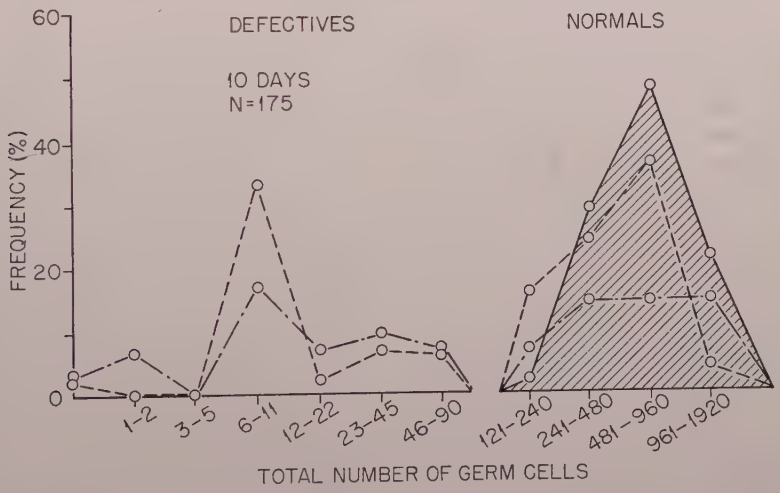

Fig. 2 Distribution of germ cell numbers in 10-day embryos segregating for genes at *W* and *Sl* loci.

		Defectives : Normals	χ^2	*P*
- — - —	Group I	52% :48%	0.74	0.30–0.50
— — — —	Group II	20% :80%	1.2	0.20–0.30
————	Group III	0% :100%		

with the C57BL/6 strain. More recently, W^v/w heterozygotes of a new strain background, WB, were obtained from Dr. E. S. Russell. This strain was developed originally in an attempt to improve, through selection and inbreeding, the longevity of W/W mice, which are severely anemic and generally die within a few days after birth (Russell and Lawson, '59). Anemic individuals showed a substantial increase in survival time on the new background although the physiological basis for the improvement could not be defined, since it was not necessarily accompanied by amelioration of the blood picture. Anemia is less severe in the W^v/W^v animal, which is viable; this allele was transferred for other reasons to the same background.

A pair of these W^v/w mice soon produced three fertile W^v/W^v sons and the familial incidence suggested a possible genetic basis for the change to fertility. Previous reports of fertility in the homozygotes indicate that it is rare (Grüneberg, '52). Veneroni and Bianchi ('57) state that it has never occurred among their control males.

It was desirable first to clarify the question of whether the W locus might be a complex one, as has been suggested, consisting of closely linked genetic units. Crossing over or mutation exclusively in the germ-cell-controlling component of one W^v/w parent might then lead to production of white, anemic progeny that would be in effect heterozygous for the germ-cell subgene, and therefore fertile. This hypothesis was examined by outcrossing the fertile W^v/W^v males to a number of W/w females. The latter were first tested with littermate W/w males, and found to produce, among their W/W offspring, only individuals markedly deficient in germ cells, as ascertained by histological study of the gonads. All white, anemic offspring (W/W^v) from the mating W/w with W^v/W^v were also sterile, as verified histologically. The gene contributed by the father therefore still transmitted a sterility effect in each case. We may conclude that there is no need to abandon the view that unity of primary gene activity at a single pleiotropic locus is involved.

It is altogether conceivable that the three kinds of cells influenced by this locus may have their response differentially altered by other factors, themselves und gene control (e.g., hormonal activit During the course of selection and inbre ing leading to development of the V strain, modifiers in some way favori fertility, possibly indirectly, may well ha become fixed. This would be consiste with the good breeding behavior shown that strain (Russell and Lawson, '59).

The other W^v/W^v adults that have sir been born have been tested for fertili Of those adequately tested to date, 15 16 males are fertile, including some deriv after outcrosses to C57BL/6, and 2 of females are fertile. The males show c tinued fertility, whereas the females ha been limited to only one small litter ea of one and four offspring. The inferen from this sex difference will be consider in Section III. Selection and inbreeding a being continued, selecting now, howev for fertility of W^v/W^v. If sufficient i provement in fertility should occur, u formly homozygous mutant litters of e bryos would be available for extendi investigation of the early etiology of t blood and pigment cell defects.

In a sample of white, anemic males th far examined histologically, the germ c population remains sparse for at leas week after birth and increases thereafte of course, prospective fertility is unknov at these ages. In marked contrast to t one tested sterile adult found (fig. 7), ma germ cells are present at reproducti age in the others examined. It is partic larly striking, however, that, despite pr ence of many normal regions, a number abnormalities are visible (fig. 8, 9). Sp matogenic elements may be lacking some cross sections of tubules, which m have been too distant from the few ava able proliferating stem cells (see Secti IV). In other areas, however, where sp matogenesis has clearly been occurring, may be arrested or abnormal. Germin cells are sloughed off into the lumen fro the basement membrane, vacuolated pycnotic spermatocytes occur, and broke off sperm tails are found in the tubules

Production of anomalous spermatoge stages may be a delayed expression of t same gene effect that, in the sterile, e treme cases, impairs proliferation of p mordial germ cells.

From resemblance of the histological cture to the lesions observed by Katsh d Bishop ('58) in induced aspermato- nesis in the guinea pig after injection guinea pig testis plus adjuvant, an ternative possibility suggests itself. ıtsh and Bishop postulate that the in- ɔduced testicular material may become ıtigenic under these circumstances and rve to trigger in the host an immune re- onse that is also directed against the ɔst's own spermatogenic tissue. The le- ɔns in the fertile W^v/W^v mouse testis ight represent an autoimmune response sulting from a complete reversal of the ɔrmal developmental sequence whereby rm cells become numerous during em- yonic life, before maturation of the ani- al's own immunologically competent tis- e. Billingham ('58) has discussed the ɔposition that potential antigens may ed to be formed sufficiently early in velopment so that the organism can be- me "tolerant" of them before his immune sponse matures. Whether or not tardy rmation of sufficient spermatogenic units rves to set off autoantibody production the mutant male mouse remains entirely be tested experimentally.

III. FURTHER GAMETOGENESIS IN THE FEMALE FETUS

There has been a controversy of some years' standing on whether the defini- ve oocytes of the mammalian ovary come ɔm germ cells set aside in the embryo, or ɔm proliferation and differentiation of e ovarian epithelium (Brambell, '27; verett, '43). The chief arguments in vor of the latter view are presented else- nere (Mintz, '59) and will be dealt with ly briefly. They emphasize the physical esence of oocytes in the outer layer of e adult ovary, and the occurrence of itotic figures in the epithelium. The rly prophase figures of meiosis are gen- ally acknowledged to be absent from the stnatal ovary; this has generated specu- ion that synapsis of homologous chro- ɔsomes is lacking or is cytologically ıorthodox and hence undetectable in ammalian oogenesis.

The evidence from mutant mice shows at a pronounced deficiency of primordial rm cells in embryonic life leads to a erile ovary in the adult. The epithelium of these ovaries does not lack the capacity for mitosis: in the W^v/W^v adult there is excessive epithelial proliferation, possibly resulting from hormonal imbalance, and leading to tumor development (Russell and Fekete, '58).

In order to learn whether the ovarian epithelium might require some stimulus from embryonic primordial germ cells before it could itself succeed in forming oocytes, chimeric associations were arranged *in vitro* so that mutant ovary would develop in contact with some of the primordial germ cells of a normal gland. Halves of ovaries were placed together on the surface of the agar medium introduced by Wolff and Haffen ('52), at 34°C. These quickly fuse. The mutant pieces ranged in age from 13 days of fetal life to 9 days postnatal; the normal ones were taken from 13- to 19-day fetuses. In no case did the epithelium of the mutant, after as many as 8 days of culture, form germ cells.

In normal embryos, there is scarcely any need to postulate postnatal neoformation of oocytes, as the total number of germ cells at the end of migration is far in excess of all future functional needs for the entire reproductive life of the female (fig. 10). As for location of germ cells in the epithelium, it is not uncommon for some of them to be situated here even when they first enter the germinal ridges in the fetus.

Early meiotic prophase stages are missing in the postnatal ovary because all the female germ cells enter meiosis before birth. The same germ cells that show the phosphatase reaction during the migratory period continue their development into meiosis while they are still phosphatase positive. Leptotene has already started in some at 13 days. This is independent of hormone influences from sources extrinsic to the sex glands, as it can start and progress in ovaries explanted *in vitro*. If factors within the female gonadal environment initiate the sudden transition from mitosis to meiosis, then the extragonadal origin of germ cells serves the useful function in the female of enabling germ cell number to augment markedly before mitotic activity declines. Primordial germ cells of the mouse look identical in both sexes and they

do not pass through any distinctive "oogonial" stage in the female before meiosis begins. Leptotene and zygotene stages are common in the early fetus; pachytene oocytes are numerous at 16–17 days (fig. 11). They may continue through diplotene and diakinesis; many are in the dictyate stage by birth and remain so, though they continue to increase in size, until just before ovulation, when the chromosome organization is again visible and anaphase and telophase of meiosis I are completed.

In the preceding discussion of improved fertility in W^v/W^v mice of the WB strain, presumably owing to fixing of genetic modifiers, it was pointed out that the improvement occurs chiefly in males. This difference is easily understood if we realize that increase in germ cell number in the female can occur only before meiotic prophase sets in. If conditions favoring germinal proliferation are slow in operating, the female is at a marked disadvantage as compared with the male.

Final experimental confirmation of the continuity of the female germ line, and of failure of somatic cells to contribute to the definitive oocyte population, would be provided if individual germ cells in the embryo could somehow be identified and shown to survive to a functional age, and if cells resulting from somatic mitoses in the adult ovary could similarly be recognized and found not to become oocytes. Such evidence is now available as a result of recent work by Dr. George Rudkin (The Institute for Cancer Research, Philadelphia. He has kindly permitted me to cite these unpublished data). He has introduced tritiated thymidine by intraperitoneal injection into pregnant female mice at 12–15 days' gestation, when some primordial germ cells in the female fetuses are still in a premeiotic state. The isotopic compound is rapidly incorporated into DNA being synthesized in various kinds of cells. In fetuses sacrificed at 17 days' gestation, some oocytes are seen, in autoradiographic preparations, to be labeled. After similar prenatal treatment, females maintained alive until 6 weeks after birth still had labeled oocyte nuclei, including some in well-developed follicles. The ovaries of the injected mothers from the same series have not yet been examined, but we would anticipate, from the observatio of Sirlin and Edwards ('59), that rad activity will not appear in their oocytes. these experiments, C^{14}-labeled adenine w used; when injected at 2–6 weeks af birth, it failed to show incorporation i oocyte nuclei, even when pregnant ma serum was simultaneously administered.

Since synapsis starts fairly synchr ously in the female, any defect in init chromosome pairing leading to abnorm karyotype in offspring from those game would show no dependence on matern age. Meiosis in the human female a starts before birth (Witschi, '56, p. 4 An extra autosome is now known to associated with monogolism in the huma and mongoloid births are more freque from mothers of advancing age (see lite ture summary in Warkany, '60). T fault leading to trisomy is therefore n likely to have occurred earlier than a phase of meiosis I. Neither a mitotic er during the primordial period nor an er in meiotic pairing in the mother's oocy would be implicated, since both oc within a brief period in all germ cells.

The germ cells in the mouse embryo a fetus may be damaged or destroyed exposure to X rays (Mintz, '58, '59). W the degree of current interest in biologi effects of radiation, particularly on ger inal tissue, clarification of normal game genesis seems essential for evaluation hazards in exposure of germ cells p natally. From normal oogenesis, we wou expect that some replacement of germ ce destroyed by radiation might occur p meiotically only, provided that enou primordial cells remained intact. If t ovarian epithelium instead were resp sible for production of new oocytes, su stantial regeneration should still take pla despite depletion of the store of primord germ cells.

C57BL/6 embryos were given a total 400 r of X rays at 8–12 days (*in utero*, whole-body exposure of the mother to 75 day, except for 100 r on day 10), duri germ cell migration. By 14 days, each h less than 100 germ cells, including so that were clearly necrotic; a few fetuses fact no longer had any germ cells at a After similar treatment, and autopsy at days, ovaries (fig. 12) resembled those

e homozygous sterile mutants (cf. fig. 5). ter observations were made after *in ro* culture of such 19-day gonads be- use of death of the individuals neo- tally. At the chronological equivalent of week of postnatal age, no improvement germ cell complement could be seen, hough somatic tissues remained viable. Certain general cautions might be men- ned in connection with investigation of mparative radiosensitivity of germ cells different pre- and postnatal ages. First, gative breeding tests alone do not neces- rily demonstrate that total destruction of rm cells has taken place after irradiation embryos. Developing accessory struc- es and ducts may have been adversely fected. Or, a minimal number of ovarian licles might be required for favorable rmone balance and fertility. Second, fferences in fertility after irradiation be- e and after birth with a given dose may t reflect only differences in radiosen- ivity of the germ cells, since capacity of rviving female germ cells to proliferate d replace lost ones terminates at onset meiosis. Spaced histological observa- ns are therefore required in conjunction th breeding data to ascertain actual mage as well as degree of subsequent covery (Russell *et al.*, '58; Ingram, '58). ially, treatment at different ages may volve only a single germ cell stage in one se but a mixture of stages in another. fore 13 days in the mouse embryo, all rm cells are in the "primordial" phase, ough they may be in different parts of mitotic cycle at the time of exposure. ese cells afterward comprise a progres- ely smaller part of the total, are less ely to be in mitosis, and are accom- nied by increasingly advanced meiotic ges. Oakberg ('58) has already pointed that an advantage in studying radia- n response of the adult ovary is that rm cells then are primary oocytes uni- mly in dictyate except for those close ovulation.

According to the same author, after 50 r X rays, all younger oocytes in the adult use ovary are destroyed, and although re mature follicles are more resistant, latter decline in number owing to nreplacement from younger stages, and parent failure of formation of new oo- cytes. After this relatively low dose, the litter number per female was reduced from an average of 14.4 to 4.0, before permanent sterility set in; productivity was reduced to 1.7 litters after 200 r (Russell and Freeman, '57).

By contrast, although sections of embryos made 2 days after they had received 200 r at 11 days show that numbers of germ cells have been damaged, some primordial ones are intact (Mintz, '59), and fertility in a small sample grown to adulthood has surpassed five litters thus far, in each case.

Although radiation damage to a given fraction of adult oocytes may be more serious, from the point of view of failure of replacement, than to embryonic primordial germ cells, it is nevertheless in the earlier period that induced point mutations may become established as clusters if the affected cell continues to proliferate. In cases of spontaneous gonadic mosaicism (see Grüneberg, '52, p. 525), the mutated sector might be clonally derived from a single primordial germ cell in the embryo.

IV. FURTHER GAMETOGENESIS IN THE MALE FETUS

The dynamics of postnatal spermatogenesis have been greatly clarified by the studies of Clermont and Leblond ('53) on mode of renewal of spermatogonia. They have demonstrated that, of several types of spermatogonia (A, intermediate, B) the primitive A type serves as a stem cell, perpetuating its own kind while also cyclically giving rise to successive generations of spermatocytes in the adult. Their investigations on the rat and other species have been extended by Oakberg ('56) who has analyzed the details of the spermatogenic cycle and of germ cell renewal in the adult mouse. Oakberg ('55) has also been able experimentally to confirm the stem-cell role of the type A spermatogonium through irradiation studies in which repopulation of the tubules was shown to depend on the proliferative activities of remaining cells of this kind.

It appears, therefore, that contributions from somatic cells need not be invoked as an explanation of gametogenesis in the male. The question still requiring elucidation is: What is the relationship between the primordial germ cell in the male em-

bryo and the type A spermatogonium of the adult? Many workers have claimed that the former disappears and that the supporting cells of the tubules then give rise to spermatogonia.

Clermont and Perey ('57) investigated this transition in late fetal and early postnatal stages of the rat and found that the primordial germ cells (which they also call gonocytes) are the sole germinal elements in the fetus and shortly after birth. As in the mouse, they have a lightly staining spherical nucleus with finely dispersed chromatin and at least two round nucleoli; the cell membrane is distinct. Their number declines, and at 4 days of postnatal age some spermatogonial A cells first appear. Quantitative study of the cell population in the tubules led to the conclusion that the primordial germ cells directly proliferate the postnatal spermatogonia.

This conclusion is fully supported experimentally by our evidence from irradiation of mouse embryos. After migration, the primordial germ cells in the mouse become enclosed in developing medullary cords that are easily visible in the gonad of the 13-day fetus. The germ cells continue to multiply and are seen occupying the center of the tubules in the fetus (fig. 13). By late fetal life, they may perhaps be in a transitional or "pre-A" state; alkaline phosphatase activity is less pronounced histochemically and mitotic figures are rare. After this time, many enlarged and degenerating ones are seen. The first type-A spermatogonia appear at 3 days after birth; they increase in number thereafter, and primordial germ cells quickly decrease. The latter are gone by 1 week after birth. Before that time, the more advanced types of spermatogonial cells are already present. Postnatally, the spermatogonia lie peripherally in the tubules, against the basement membrane. The A type differs from the primordial cell chiefly in having slightly coarse chromatin particles adherent to the surface of the nucleoli.

When male embryos were irradiated *in utero* with 400 r of X rays at 11 days of gestation, the tubules at 19 days still contained a fair number of germ cells. When the same total dose is distributed in five parts at 8–12 days of embryonic life (75 r/day, except for 100 r on day 10), only exceedingly few germ cells are present at 19 days. Since these mice do not survive after birth, the gonads were explanted long enough to observe whether the postnatal population of A-type spermatogonia would or would not appear. They did not (fig. 14), although some mitoses of supporting cells were visible. Proliferation of A cells did occur in unirradiated control gonads explanted to the same kind of agar medium at 19 days' fetal age. Failure of the adult kind of spermatogonium to form after virtual depletion of migrating primordial germ cells in the embryo points to the conclusion that, in the male as in the female, the germ line is continuous from embryonic to fetal to postnatal life.

Regenerative capacity is of course greater in the male than in the female line, since it is retained as long as the spermatogonial stem cells function. In both sexes, the early history of the embryonic germ cells is clonal, but the clone is a shorter one (less than 10 cycles of division) in the female. Clusters of mutated male germ cells may also originate in the clonal period, which extends beyond 10 divisions until the stem cell renewal pattern is introduced. Incidence of spontaneous mutation in adult spermatogonia might differ from that in embryonic germ cells because of the difference in temperature at which they develop, and a single A cell would replicate errors in a smaller fraction of the total gamete population than would a single migrating primordial cell.

CONCLUSION

Many elusive problems concerning gametogenesis remain to be investigated. Included are: origin of the germ cells found in the yolk sac; mechanisms guiding migration to germ ridges; meiogenic influences promoting synchronous onset of meiotic prophase in oocytes; mechanisms of meiotic pairing; contributions of neighboring somatic cells to physiology of developing gametes, and influence of genetic factors on this; differentiation of germ-cell-specific synthetic properties; nature of gene action on germ cell development; and control of germ cell population size and cyclical maturation.

OPEN DISCUSSION

SILVERS[4]: The autoimmunity explana- n is very intriguing and one that cer- nly deserves testing, especially since it uld be very easy to test. As far as the erance responsive period of an animal concerned, it depends on the antigen in estion. For example, in the mouse, the erance responsive period—with respect strong histocompatibility antigens (e.g., 2)—is over at or shortly after birth. wever, with weak histocompatibility an- ens such as that apparently determined a gene(s) on the Y chromosome, the erance responsive period is greatly pro- ged. Dr. Billingham and I have found t 60% of C57BL/6 females can be dered tolerant of this "Y antigen" after ection of isologous male spleen cells as e as 17 days after birth. It seems to me refore that, since the antigens with ich Dr. Mintz might be dealing (i.e., oantigens) would almost certainly be aker than our "Y antigen," we might pect the tolerance responsive period to even longer.

MINTZ: I cannot, of course, say any- ng as yet about the exact time at which histological picture changes, approach- the normal number of cells here.

E. S. RUSSELL[5]: I like tolerance, and I o like pleiotropism very much. I think, vever, from my point of view it seems re probable the deficient spermato- esis is a part of the *W* series pleiotro- m of biochemical effects, which happen work on germ cells and blood-forming ue and pigment-forming cells, rather n an autoimmunity.

t is a question here, regarding germ s, whether the females or the males are special case. I think maybe female m cells are, because they reach the yte stage before birth, so nothing more happen to them. Maybe the male germ is more like erythropoietic tissue or lanoblasts. Let's now go on talking par- ularly about erythropoietic cells. In all viable *W* genotypes, blood formation st go on. It goes on abnormally. It s not achieve as high a hematocrit level in a normal mouse. It goes on more wly, but it goes on.

n this particular stock in which Dr. ntz found the fertile males, and I find them too, perhaps the selection, favoring a sufficient amount or a sufficient rate of erythropoiesis to keep *WW* alive as long as possible, has also collected modifiers that will promote survival or passage of a larger number of the few remaining spermatogonia from, say, type A to type B so that they can undergo spermatogenesis. But there still is a deficient number of them, and a much smaller number will get to the B stage. Thus where you have a tubule that has undergone some spermatogenesis, as evidenced by the sperm you saw in the tubule, it may have exhausted its supply of cells able to go through this slow and difficult stage from A to B. The difficulty may be attributable to biochemical defects similar to the defect that keeps early erythroblasts from going easily to the later stages of erythropoiesis.

Just one more thing about the erythroid biochemistry—we now have evidence that the type of erythropoietin usually used in experiments, which stimulates normal animals to extra erythropoiesis, also stimulates normal mice in this way, but has no influence at all upon W/W^v or W^v/W^v mice. They are completely resistant to this stimulus. However, there are other stimuli, such as oxygen deprivation, which Grüneberg found long ago, and which we have confirmed, to which the anemic and the normal mice react in the same way. This is localizing the effect of the defect in the erythroid tissue to a stage at which erythropoietin is a stimulus to proliferation of erythroid tissue.

I feel that all these aspects of *W* pleiotropism will work out to be a part of one single biochemical pathway, or of related biochemical pathways acting in different tissues.

WAELSCH[6]: I would like to go back to an earlier stage that you discussed. First of all, do you completely discount an inductive effect of the promordial germ cells on the later differentiation of germ cells from the germ cell epithelium. I think this might perhaps be difficult to exclude.

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[4] W. K. Silvers, The Wistar Institute of Anatomy and Biology.

[5] E. S. Russell, Roscoe B. Jackson Memorial Laboratory.

[6] S. G. Waelsch, Albert Einstein College of Medicine.

In that connection have you, with the help of the new, more viable and more fertile males, studied any embryonic material to see whether there is actually an early suppression of primordial germ cells and whether perhaps more germ cells migrate and more become lodged in the gonadal ridge of the modified than of the unmodified strain?

I have one final remark. You mentioned that the *W* effect on germ cell formation was the earliest genetically determined embryological effect observed. I would like to remind you that Dr. L. J. Smith, who is here, has to my knowledge described the earliest known embryonic effect in one of the *t* alleles in t^{12}, where the effect has actually been demonstrated to take place before implantation; some of the other *t* alleles also have effects perhaps preceding the one you described but at least occurring at the same time.

MINTZ: Yes, I am acquainted with Dr. Smith's interesting work on the *t* alleles. My remark about the earliest effect on embryonic development being the effect on primordial germ cells was intended simply to emphasize that these cells are, in a manner of speaking, the earliest stage of the *next* generation. In this sense, the gene effect on them precedes matters that occur after gametes mature, participate in fertilization, and give rise, in time, to blastocysts, etc.

As to your question regarding earlier germ cell numbers in these fertile individuals, so far as the evidence at present indicates, the earlier number in them is subnormal. I would like for my own satisfaction, however, to get more information on that topic.

Concerning your other point, it might be admissible that the earliest germ cells might have an inductive effect on activities of the epithelium. I have tested this experimentally by confronting *in vitro* a portion of an embryonic normal ovary, containing a full complement of germ cells, with a piece of a mutant ovary such that, in regions of fusion, normal primordial germ cells often come to lie in contact with the epithelium of the mutant. This in no case elicits the slightest gametogenic activity from that epithelium.

PILGRIM[7]: Do you have any eviden that would indicate whether it is the ge tics of the germ line or the genetics of t somatic cells that will determine wheth an animal has a testis or an ovary?

MINTZ: Experimental sex reversal h been used in the past to examine th question. In studies on various vertebrat but especially amphibians, it has appear that the primordial germ cell, regardless its genotype, is sexually bipotential. development may be directed into the o posite channel, but breeding experimen after sex reversal demonstrate that its s chromosome constitution has remained tact. As for the somatic cells of the different gonad, many studies by Witsc and others have shown the importance genetic control in setting a balance tween prospective female (cortical) a male (medullary) components, althou nongenetic factors may sometimes distu this balance. There is, then, an influen on germ cell development within the major parts, and possibly some recipro interactions are at work.

SILVER: You have so beautifully de onstrated that, in so far as the germ c abnormality of the *W* locus is concern it can be traced back to the 8th to 12 day of gestation. I think it is of inter that this is exactly the same period which melanoblasts are migrating fr the neural crest to their definitive po tions. Indeed, I would not be surprised observe a similar story with respect to t melanoblast if a melanoblast-specific str was available. Would Dr. Russell or y have any ideas, since these two systems up so well with each other, whether th is any hope for tying in the erythropoie effect during this time?

E. S. RUSSELL: I think perhaps y since it is already well established at days, the probability is it was there befo The use of isotopes is the only way I c see of getting at it.

RUNNER[8]: I am sure that most of realize it, but lest the scholarly summa presented by Dr. Mintz leave an impressi that only germ cells of the embryo responding to the dose of X ray used

[7] H. I. Pilgrim, University of Buffalo School Medicine.

[8] M. N. Runner, National Science Foundatio

nderstand around 400 r), I wish to point ut that this is about four times the dose equired to produce abnormal morpho- enesis in other tissues in the same em- yo.

Since irradiation at 9 and 10 days post- pitum severely affects many types of cells the embryo in addition to the germ cells, may be permitted to suggest that effects X ray in tissues adjacent to and sur- ounding the surviving germ cells may fluence their subsequent history. This ould lead to quite a different interpreta- on about the possibility for postnatal ultiplication of germ cells in the female.

MINTZ: Drs. L. B. Russell and W. L. ussell have shown in very extensive stud- s that other defects are involved. This es not change the facts that one observes the case of germ cell development. any primordial germ cells are killed by ses considerably below 400 r. There is particular reason to think that function all epithelial cells is impaired by doses at eliminate germ cells.

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PLATE 1

EXPLANATION OF FIGURES

3 Germinal ridge of 11-day female embryo, stained for alkaline phosphatase; germ cells at periphery. × 160.

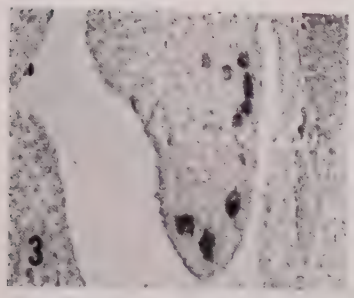
4 Germinal ridge of 11-day male embryo, stained for alkaline phosphatase; germ cells in center. × 160.

5 Ovary of W^j/W^j mutant 3 days after birth; deficient in germ cells. × 170.

6 Testis of newborn W/W mutant; deficient in germ cells. × 115.

7 Testis of W^v/W^v sterile male 10 months old, with germ cells absent and friable PAS-positive material in lumen of tubules. × 70.

8 W^v/W^v testis at 10 weeks of age; many abnormal spermatogenic cells. × 220.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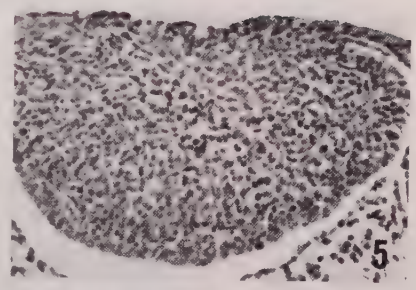
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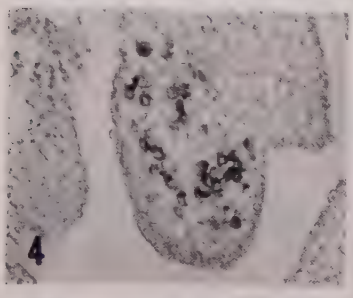
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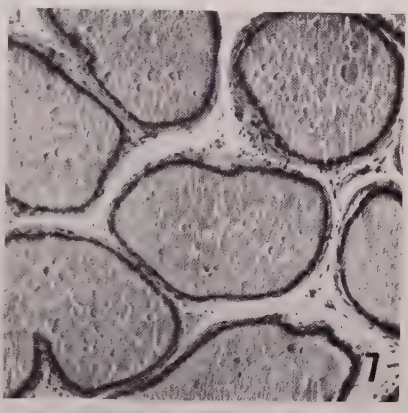
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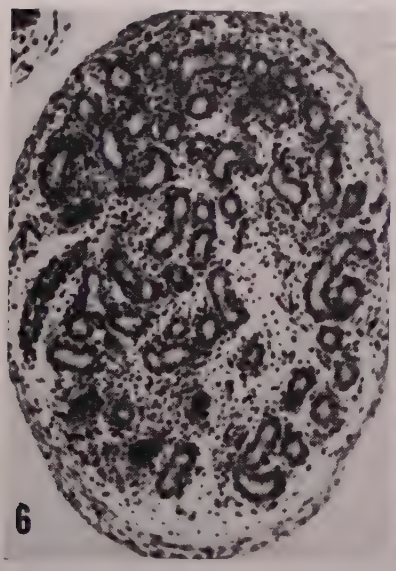
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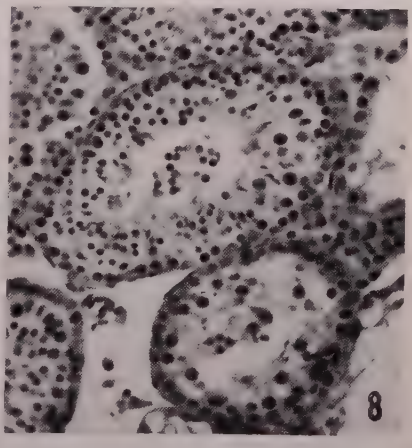
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PLATE 2

EXPLANATION OF FIGURES

9 W^v/W^v testis at 19 weeks of age; many germ cells present, including abnormal stages. × 110.

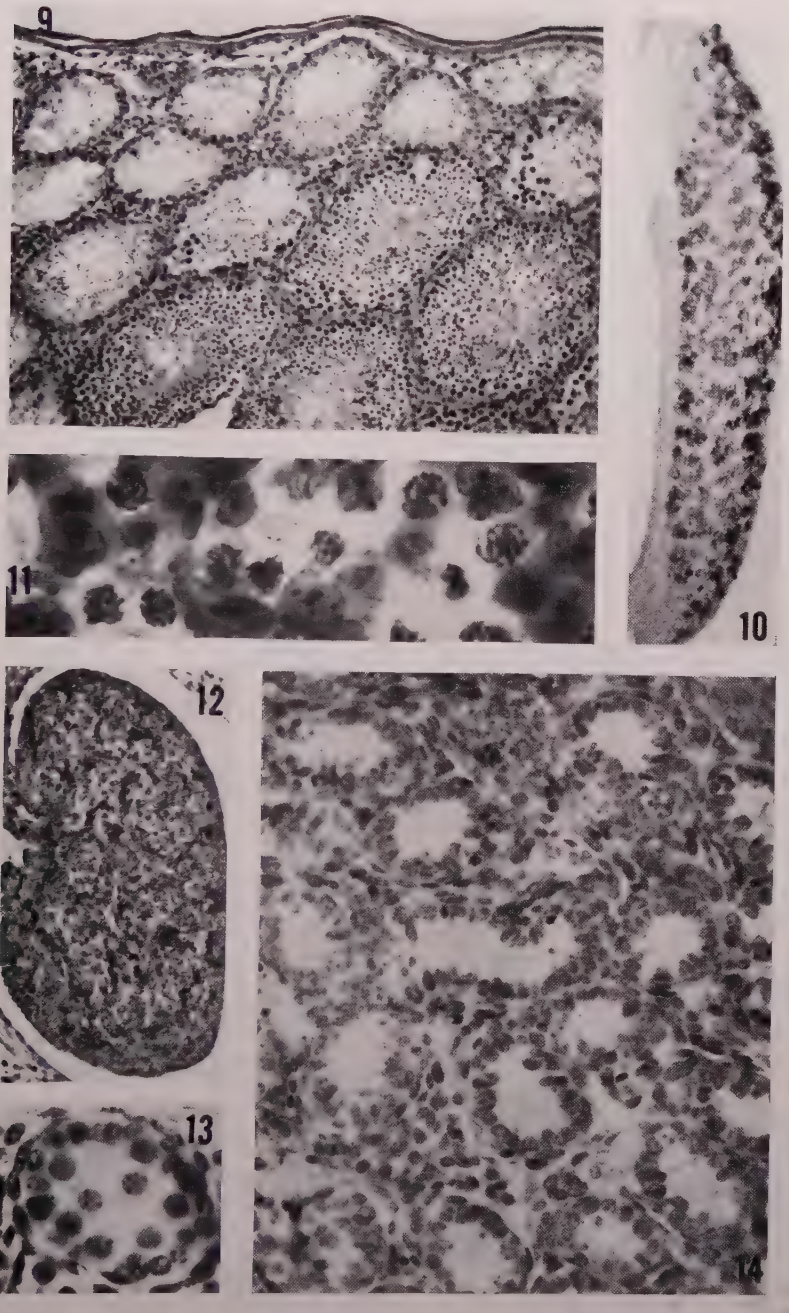
10 Ovary of 14-day fetus, stained for alkaline phosphatase; germ cells very numerous. × 110.

11 Ovary of 17-day fetus; note pachytene oocytes. × 865.

12 Ovary of 19-day fetus after irradiation with 400 r total at 8–12 days of embryonic life; deficient in germ cells. × 160.

13 Testis of 17-day fetus; germ cells in center of tubules. × 540.

14 Testis explanted *in vitro* at 19 days of fetal life after irradiation with 400 r total at 8–12 days; spermatogonia absent after 4 days in culture. × 385.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evelopmental Genetics in the Mouse, 1960

HANS GRÜNEBERG
University College, London, England

The development of mutants in the ouse has been studied mainly for three fferent reasons. From a purely genetical int of view, the material can be used r studying the mechanisms of gene ac-on. From the embryological point of ew, it can be used for studying embryo-gical mechanisms in a group not readily cessible to experimentation. And from e medical point of view, it represents tually the only material available for e study of the pathology of development. ot being primarily an embryologist, I el that the assessment of progress in lation to embryology had better be left a representative of that field. This sur-y is devoted to the question of how far velopmental genetics in the mouse has ogressed toward an understanding of ne action; it is mainly designed to show me of the gaps and the present limits the field. This will lead to a discussion some of the ways along which further vances may be possible in the future. few words on the relation of develop-ental studies in the mouse to medicine ll be added at the end.

For reasons of time, it will obviously be possible to deal with all the mouse nes that have been described. To nar-w down the field to a more manageable e, we can broadly divide the genes of e mouse into two categories. In the first these are the genes with an effect in rly embryonic development that leads a stable end result. For instance, the nes for vestigial-tail (*vt/vt*) and for ndactylism (*sm/sm*) lead to regression the tail and to fusions between adjacent gits, respectively. There is no reason to ppose that, when this has happened, e genes in question have any further ects on these deformities as such. The uation is quite different with genes that e responsible for continuing processes. r instance, the genes for brown fur (*b/b*) and for macrocytic anemia (W^v/W^v) lead to the production of brown rather than black eumelanin and of macrocytic erythrocytes, respectively, as long as the mouse lives. There is thus at least a prima facie case that the genes in question continue to be active in the melanocytes and bone marrow cells, respectively, throughout life. In this survey, I have confined myself to genes falling into the first of these two categories. I am, of course, well aware that the distinction made here for practical purposes is probably not a fundamental one, and in several instances, the inclusion of a gene in one rather than the other category has been somewhat arbitrary. I shall come back to this point in some detail later in this discussion.

As a basis for discussion, I have assembled, in table 1, 42 entities for which embryological data are available. The genes for hydrocephalus-1 (*hy*-1/*hy*-1) and for shaker-short (*st/st*), both of them extinct, have been omitted because of the criticisms (Grüneberg, '52) to which the embryological evidence has been submitted. I have also omitted the genes for shaker with syndactylism (*sy/sy*) and for Oligosyndactylism (*Os*/+) since the embryological information (Grüneberg, '56) is still incomplete, and those for screw-tail (*sc/sc*; Bryson, '45) and phocomelia (*pc/pc*; Fitch, '57) where only part of a complex syndrome has been studied embryologically so far. In the latter four cases it is virtually certain that further work will trace the gene effects to a significantly earlier stage of development. In table 1, the genes are arranged roughly in the order in which their first known effects appear in development. The time scale of these effects is only very approximate in view of well-known discrepancies between chronological and developmental age of embryos when different stocks of mice are compared. For instance, it is

TABLE 1

Early embryonic effects of some mutant genes in the mouse

No.	Mutant	Symbol	Day of onset	Embryology	Reference
1	—	t^1/t^1	$< 3\frac{1}{2}$(?)	Preimplantation lethal not yet individually identified	Gluecksohn-Schoenheimer, '38b; Smith, '56
2	—	t^{12}/t^{12}	$3\frac{1}{2}$	Reaches the morula (about 30-cell) stage but not the blastocyst stage. RNA reduced	Smith, '56
3	Yellow	A^y/A^y	5	Degeneration in the blastocyst stage (50–100 cells); implantation remains incomplete	Robertson, '42
4	—	t^0/t^0	$5\frac{1}{2}$	In early egg cylinder stage no separation of inner ectodermal cell mass into embryonic and extra-embryonic portions; entoderm abnormally thick and coarse	Gluecksohn-Schoenheimer, '40
5	—	t^{w5} group	$6\frac{1}{2}$	In egg cylinder stage lack of differentiation of entoderm and embryonic ectoderm with some pyknosis and degeneration	Bennett and Dunn, '58
6	Kinky-tail	Fu^{kt}/Fu^{kt}	7	Hyperplasia of embryonic tissue leading to duplication of organs, to formation of extra embryonic axes or to complete twinning; lethal	Gluecksohn-Schoenheimer, '49
7	Brachyury	T/T	$8\frac{1}{2}$	Probably abnormality of primitive streak. Notochord retained in gut or incorporated in neural tube. Posterior part of body greatly reduced; no vascular connection with mother	Chesley, '35; Grüneberg, '58b
8	Brachyury	$T/+$	9	Probably abnormality of primitive streak. Notochord of normal length but tends to be retained in hind gut or cloaca or both; separation from primitive streak and neural plate also delayed. In tail notochord in places incorporated in tail gut or neural tube suggesting stickiness	Chesley, '35; Grüneberg, '58b
9	Tailless	T/t^0; T/t^1	9	Similar to $T/+$, but notochord incorporated in neural tube in the whole of the tail	Gluecksohn-Schoenheimer, '38a; Grüneberg, '58b
10	—	t^{w1} group	9	Retardation, pyknosis in CNS, particularly in hind brain and in ventral aspects of brain and spinal cord; edema, vascular and pericardial enlargement; microcephaly and hydrocephaly with chondrocranial anomalies	Bennett *et al.*, '59

11	Fused	*Fu/Fu*	9	Waviness, ventral buds, and posterior duplications of neural tube; abnormal tail somites; notochord and vertebral column probably only secondarily involved	Theiler, '56; Theiler and Gluecksohn-Waelsch, '56
12	Loop-tail	*Lp/Lp*	9	Cranioschisis and rachischisis; overgrowth of neural tissues; secondary tail twist	Stein and Rudin, '53
13	curly-tail	*ct/ct*	9	Rachischisis, rarely cranioschisis; secondary tail twist	Grüneberg, '54a
14	Danforth's short-tail	*Sd/+; Sd/Sd*	9	Probably anomaly of primitive streak giving rise to abnormal notochord, which subsequently degenerates; secondary involvement of cloaca and metanephros, particularly in *Sd/Sd*	Gluecksohn-Schoenheimer, '45; Grüneberg, '58a; Theiler, '51a,b, '54
15	Patch	*Ph/Ph*	9	Edema, vascular and pericardial enlargement and subepidermal blebs of unknown origin, with cleft-face in some older embryos; lethal before birth	Grüneberg and Truslove, '60
16	kreisler	*kr/kr*	9	Ear vesicles formed too far from myelencephalon followed by defective differentiation of labyrinth	Hertwig, '44
17	Splotch	*Sp/Sp*	9½	Cranioschisis and rachischisis with overgrowth of neural tissues; spinal ganglia reduced or absent; no (potential) fur pigmentation; secondary tail twist. Disturbance of neural crest and dorsal part of neural tube postulated	Auerbach, '54
18	fidget	*fi/fi*	10	Lens reduced to about 80% from the beginning. Abortive differentiation of vestibular part of labyrinth, but not of cochlea	Truslove, '56
19	anophthalmia	*ey−1/ey−1*	10	Optic vesicle grows at reduced rate and usually does not reach the overlying epidermis to induce a lens	Chase and Chase, '41
20	tail-kinks	*tk/tk*	10	Disturbance of differentiation of sclerotomes into anterior and posterior sclerotome halves of differing tissue density	Grüneberg, '55a
21	vestigial-tail	*vt/vt*	10	Reduction of ventral ectodermal ridge of tail, a putative stimulatory organ for tail growth; also anomalies of neural tube	Grüneberg, '57
22	Crooked-tail	*Cd/+*	10	Disturbance of segmentation with small and deformed somites, which may be fused with each other	Theiler, '56; Matter, '57

TABLE 1 (*Continued*)

Early embryonic effects of some mutant genes in the mouse

No.	Mutant	Symbol	Day of onset	Embryology	Reference
23	Pintail	*Pt/+; Pt/Pt*	10	Reduction in growth rate of notochord	Berry, '60
24	syndactylism	*sm/sm*	10	Hyperplasia of limb (and sometimes tail) epidermis, including apical ectodermal ridge; overgrowth and deformation of limb buds	Grüneberg, '56, '60
25	hemimelia tibiae (luxate)	*+/lx; lx/lx*	10½	Shape of hind limb buds abnormal at 10½ days, i.e., before blastema formation; craniad shift of hind limb girdle statistically demonstrable at 9½ days	Carter, '54
26	urogenital	*ur/ur*	10½	Tail somites shorter and broader than normal and unevenly crowded together. Foreshortening of vertebrae and head; cleft palate	Fitch, '57
27	harelip and cleft palate	—	10¾	Retarded growth of maxillary processes with failure of medial and lateral nasal processes to fuse; also secondary tearing open of imperfect fusions	Reed, '33; Steiniger, '41
28	microphthalmia	*mi/mi*	11	Increased thickness of pigment epithelium of eye leading to coloboma and microphthalmus	Müller, '52
29	dreher	*dr/dr*	11	Failure of sacculus and utriculus to separate from each other; no ductus reuniens formed; either regression of ductus endolymphaticus with increased endolymphatic pressure, or compression of membranous labyrinth by excess perilymph.	Fischer, '58
				Thickening of roof of fourth ventricle leading to absence of foramen of Magendie and hydrocephalus must have arisen before day 11	Bierwolf, '58
30	undulated	*un/un*	11	Disturbance of differentiation of sclerotomes: condensations of mesenchyme cranial to sclerotomic fissures smaller than normal	Grüneberg, '54b
31	Tail-short	*Ts/+*	11	General retardation; abnormalities of neural tube and notochord	Deol, '61
32	Bent-tail	*Bn ♂; Bn/+ ♀*	11(?)	Defective growth of tail bud inferred since segments (in adult life) are smaller in size and reduced in number; no direct embryological data	Grüneberg, '55b

33	Posterior reduplication	—	(11)	Excess material ("pelvic mass") leading to ± complete duplications of posterior half of body. Origin should be traceable at least to day 9	Danforth, '30
34	luxoid	*lu/lu*	11½	Abnormal condensation patterns of mesenchymal blastemata may be causally related to anomalous formation of apical ectodermal ridge; caudal shift of pelvis; disturbances of segmentation (tail somites); increase in total number of somites	Forsthoefel, '59b
35	Springville luxoid	—	11½	Incipient polydactylism in forelimbs at 11½ and in hind limbs at 12 days; hydrocephalus	Forsthoefel, '59a
36	congenital hydrocephalus	*ch/ch*	12	Reduction in size of skeletal blastemata	Grüneberg, '53
37	droopy-ear	*de/de*	12	Widespread skeletal anomalies traceable to defective mesenchymal condensations	Curry, '59
38	preaxial polydactylism	*py/py*	12	Overgrowth of preaxial border of hind limbs traceable to stage just before blastemal chord formation	Chang, '39
39	oligodactylism	*ol/ol*	12+	Reduction of mesenchymal material, particularly on the postaxial border of the foot plates	Freye, '54
40	blebs	*my/my*	(12)	Subepidermal blebs of unknown origin at day 12. Since the syndrome is now known to include pseudencephaly, it must have a much earlier onset	Carter, '56, '59
41	short-ear	*se/se*	13	Abnormalities of mesenchymal condensations detectable at 13 days in sternum, at 14 days in annular cartilages of ear, at birth in scapha	Green and Green, '42
42	postaxial polydactylism	*tu/tu*	14	Persistence of material on postaxial border of anterior foot plate, which disappears in normal development	Center, '55

more than doubtful whether in fact the rachischisis in Splotch homozygotes (*Sp/Sp*) appears half a day later than the same anomaly in Loop-tail (*Lp/Lp*) or in curly-tail (*ct/ct*). In any case, it is now well understood that genes come into action, not at a fixed time in development, but when prompted by the specific process they control. Such a process often continues for quite a time. This is easily seen in metameric structures such as vertebrae, where a process may creep along in a craniocaudal direction for several days; and it is particularly striking in the case of short-ear (*se/se*; Green and Green, '42), where a process involving the mesenchymal skeleton can be followed in various localities for at least a week. In the fifth column of table 1, some very brief remarks are made about the nature of the earliest known embryological events in each case; on account of their brevity, no accuracy in detail will be expected. In a gathering of this kind, they will be accepted for what they are, i.e., an aid to memory. References, of course, cannot be complete and favor more-recent papers from which earlier ones can be traced.

Table 1 is not concerned with the ultimate phenotype of the various mutants and indeed not with the pleiotropic ramifications present in nearly all the mutants considered. By recognizing secondary gene effects for what they are, the retrograde analysis of mutant genes in many cases has led to the elimination from consideration of entities of no relevance in relation to the main problem. If it is now possible to focus attention on the more basic effects of mammalian genes, this is largely attributable to the drastic pruning to which the pleiotropic branches have been subjected.

Scrutiny of the early embryological effects assembled in table 1 reveals that one typical situation dominates the picture. The differentiation of a structure proceeds normally up to a certain point; then, without any obvious reason detectable morphologically, the development of the structure comes to a standstill, or is much slowed down, or is deflected into abnormal channels. This applies, for instance, to the early lethals (nos. 2–5). In later stages of development, the growth rate of the notochord may be reduced (no. 2
an anomalous notochord may be form
that tends to break down (no. 14), o
"sticky" notochord that tends to get
corporated in its neighbors (nos. 7–
In other axial anomalies, the first det
table deviation may involve segmentat
(no. 22), the differentiation of the scle
tomes into anterior and posterior scle
tome halves (no. 20), or formation of c
tain condensations of mesenchyme in
sclerotomes (no. 30). In the cent
nervous system, the first detectable anc
aly may be a failure of dorsal clos
(nos. 12, 13, 17) or growth anomal
(no. 11): in the eye-reduced growth
the eye evagination (no. 19) or increas
development of the pigment epitheli
(no. 28), in the ear normal or near-n
mal formation of the ear vesicle follow
by defective differentiation of the la
rinth (nos. 16, 18, 29). In mutants
fecting the limbs, the first detecta
anomaly may be one of shape or size
the limb buds (nos. 38, 39) before bl
tema formation, and so on, and so
This general situation dominates the p
ture, and at least three out of every f
items in table 1 clearly or probably belo
to it. Among the few items that, on pr
ent evidence, do not seem to conform
blebs (no. 40) and Patch (no. 15).
the case of blebs earlier manifestatio
undoubtedly remain to be discovered (C
ter, '59), and the same may be true
Patch. Although it would be prematu
to generalize, it appears to be true th
in the great majority of adequately studi
mutants, development proceeds norma
up to a point at which, without obvio
morphological reasons, a specific proc
fails to happen, is slowed down, or h
pens in an anomalous fashion.

Could the seemingly "spontaneous"
set of the various anomalies be attributa
to limitations in the observers? Perha
in occasional instances a reinvestigati
by a different observer might lead to
discovery of earlier manifestations.
I am convinced that the picture wo
not be changed in principle, and that
most cases what can be discovered in s
tions stained with the conventional his
logical stains has already been discover

The question arises whether, and to at extent, refinements of histological hnique could lead to progress. With y few exceptions, histochemical meth- have scarcely been used. Histochem- l methods are more specific and varied n the conventional stains, and there almost certainly instances in which ir application will help where incipient omalies cannot be established unam- uously by the traditional methods; reover, the interpretation of anomalies tectable by standard techniques will in me instances be helped. But, though the efulness of histochemical methods in ecial instances (Bennett, '56; Smith, ; Mintz and Russell, '57) has been oved and will probably be extended to ner cases, we may well doubt whether ir use is likely to change the picture ndamentally. Compared with the spe- icity of gene action, that of histochem- l methods is bound to be rather a blunt l.

So far as I am aware, the electron croscope has not yet been introduced o the study of the development of mouse utants. And for good reasons. As in ronomy, the most powerful instruments nnot be brought to bear until the exact ot of interest has been accurately pin- inted by more modest methods. Perhaps s state has now been reached for cer- n cells in Corti's organ of the deaf ltzer-shaker mutants, which are outside e scope of the present discussion.

It seems that, for most of the mutants lividually as for the problem of gene ion in general, the retrograde analysis means of morphological methods will t carry us much farther than it has ne. That such a point would be ched sooner or later was foreseeable. in normal development new structures se epigenetically without visible, mor- ological causes, it is inevitable that the t pathological structure caused by a e must come into being in a similar nner; for, being abnormal, it cannot ve been caused by something that was lf normal. Only a preformationist uld expect to be able to trace a gene ct back all the way to a pathological unculus, or, more accurately, mus- us, in the fertilized ovum. The end of the road being thus in sight, we have to ask ourselves—where do we go from here?

The over-all picture that emerges from table 1 is that, for any one mutant gene, development proceeds normally up to a point when a specific process is disturbed. Let us consider one more example that, in its very simplicity, may point the way toward an understanding of the rest. In normal development, eye pigmentation is first laid down at the 11-day stage. From that moment onward, albinos can be distinguished from normal embryos. Albinism thus comes into being, as a visible entity, in exactly the same way as the mutants in table 1. Development proceeds normally until the point is reached when, unlike the normal, the albino embryo is unable to carry out some step in melanin synthesis. A biochemical defect is thus made manifest abruptly as the mutant embryo is first confronted with a specific task. In this case, we know the substance that the albino cannot make. It does not seem unreasonable to suggest that the mutants in table 1, or most of them, are similarly unable to synthesize certain substances that are necessary for the completion of certain specific steps in development but that, unlike melanin, have not yet been identified. It may further be suggested that these hypothetical substances are mostly proteins (and perhaps other types of macromolecules).

Recent progress on the borderline between embryology and serology should make it possible to put this hypothesis to the test. In a remarkable paper Konyukhov and Lishtvan ('59) have, by means of a gel diffusion technique, investigated the development of the lens proteins in the chicken. In the adult lens, seven distinct antigens can be identified. These come into being one after the other during development. The first appears in the invaginating lens placode (23–25 somite stage), two more in 3-day-old embryos, another two in the 4-day stage, the sixth in the 5-day stage, and the seventh and last in the 6-day stage. Most of them are thus first formed during a period of high morphogenetic activity (separation of the lens placode from the ectoderm and formation of the primary lens fibers), and it seems that antigenic differentiation pre-

cedes morphological differentiation. Presumably, a similar epigenetic sequence of lens proteins exists in the mouse. Nor does it seem too sanguine to hope that comparable arrays of tissue-specific or organ-specific antigens will be detectable in other structures. If it could then be shown that, in particular mutants, specific antigens are absent or abnormal, a real break-through would have been achieved into the wide-open country of protein chemistry.

It is tempting to speculate on how such a situation could be exploited. To mention only one possibility, we would have a sensitive tool to answer the question of whether the morphological analysis has reached the final process. For instance, if serological analysis established the existence of, say, four specific proteins in the notochord of the mouse, a gene that interfered with one of them might reasonably be regarded as affecting the notochord directly. If, on the other hand, a structurally abnormal notochord were found associated with the presence of all four specific proteins, one might conclude that the notochord is only secondarily involved.

In the introduction to this survey, a distinction was made between genes leading to essentially stable situations early (i.e., congenital defects) and genes that seem to have continuing actions throughout life. With few exceptions, the congenital defects in the mouse have indeed been treated as if they had a genetic past, but no genetic present. Yet, on reflection, we wonder whether in fact the congenital defect is the whole story or only the most conspicuous part of a more complex situation. Consider, for instance, some of the early lethals. The t^{12} homozygote dies in the morula stage; yet, when the gene survives in the T/t^{12} compound, it displays a much later effect in reinforcing the action of *T*. Similarly, whereas the yellow homozygote dies in the blastocyst stage, the gene surviving in heterozygotes has a striking and continuing effect on coat color. It must be presumed that gene action is essentially the same in homozygotes and heterozygotes (though, of course, different in degree); if so, these genes come into play more than once.

More directly this is shown by a gro
of genes whose inclusion in (or exclus
from) table 1 is rather arbitrary. For
stance, microphthalmia (*mi/mi*) was
cluded on account of its embryonic eff
on the eye; however, the failure of b
absorption that these animals show see
to be a continuing effect. (Grey-leth
gl/gl, which has no known "burnt-o
effects, but only continuing ones like c
color and the disturbance of bone abso
tion, was left out.) Perhaps more strik
is the situation in the *W* series. The s
ility of the various homozygotes is due
an early defect of the primordial ge
cells (Mintz and Russell, '57), which
clearly a closed chapter. Similarly,
spotting, being caused by the absence
melanocytes from the hair follicles
certain areas, however it may have ari
(Silvers,'56), is a once-and-for-all af
that evidently does not require continu
gene action for its maintenance. But
macrocytic anemia in these animals
sists throughout life and is clearly co
tioned by a continuing gene effect. Exa
the same situation occurs in Steel
Bennett, '56). The gene for short-
(*se/se*) has been found to reduce the
of proliferation of the osteogenic cells
the periosteum after fracture in adult m
(Green, '58) in addition to its embryo
and early postnatal effects. Perhaps
most striking illustration is the gene
diminutive (*dm;* Stevens and Macken
'58); here abnormalities of the axial sk
ton that will probably be traceable to
9-day stage coexist with a life-long ma
cytic anemia.

There is thus certainly a group of ge
that combine once-and-for-all congen
defects with persisting pathological p
esses for which continuing gene actio
the simplest interpretation. The quest
clearly arises of whether comparable
tinuing processes of a less conspicu
nature may not be present in some co
tions hitherto regarded as congenital
fects pure and simple. This leads to
fundamental question of whether
genes that control embryonic deve
ment are separate from those which con
the working of the adult organism, o
what extent genes may serve a dual
pose. The screening of mutants for

esence of comparatively mild anomalies adult life will require the application of sts of a very unspecific kind that can be rried out with a minimum of skilled por. It may be assumed that most anmalies will tend to reduce the lifean of affected individuals; similarly, the dy weight will tend to be influenced by any quite dissimilar agencies. It may erefore be suggested that the construcon of life tables of an array of mutant nes (each treated separately and with appropriate simultaneous controls), together ith monthly weighings of the animals, ay lead to detection of unsuspected conuing effects in some mutants hitherto garded as simple congenital defects. nce the existence of such an effect has en established, its eventual identification ould not be too difficult, and the study such effects is likely to deepen our derstanding of both the action of genes d the significance of congenital defects, cluding those in man.

This brings me to my final remarks out the bearing of the study of mutant nes in the mouse on medicine. Confining rselves to congenital defects, as we have ne, the study of the development of ouse mutants is the nearest medicine can t to an understanding of the pathology development. In relation to the theory gene action, our task is limited in that e shall not have to continue indefinitely examine new cases once the problem as been solved in principle. In relation to edicine, every new case is of interest and quires analysis as long as it is similar to nditions known in man. The progress ready made in this field is impressive, d every valid embryological study is an portant building stone in the edifice of e pathology of development.

OPEN DISCUSSION

MINTZ[1]: Your proposal, Dr. Grüneberg, uld certainly yield interesting informaon about a number of mutants. Perhaps e should, at the same time, bear in mind difficulty that already exists with respect "antigens" studied in normal embryos, mely that of interpreting the role of such bstances in development.

GRÜNEBERG: I certainly do not underestimate the difficulties of interpretation that may arise, and I am far from suggesting, without having tried, that all will be plain sailing. However, I hope that some of the obscurities surrounding the role of antigens in normal development may be overcome if, for instance, it is shown that a specific antigen present in the normal embryo is reduced or absent in one of the mutants.

SEARLE[2]: I would just like to point out that I think very often one would expect that genes, even if they do have what can be considered once-and-for-all effects, will at the same time have long-continued consequences. I am working with what can be considered a luxoid gene, which also removes the spleen entirely and reduces the size of the stomach. I suppose these are once-and-for-all effects, but I have no doubt if I constructed life tables and weighed the mice at regular intervals, I would find also long-continued consequences of these once-and-for-all effects.

GRÜNEBERG: I am afraid I did not make this sufficiently clear. If we try to test genes for the existence of hidden continuing effects, in the first instance, I think we should exclude genes that have a clear pathology that would in itself account for possible effects on the lifespan or weight; such as, in your case, the reduction of spleen and stomach.

SETO[3]: I don't know if it is appropriate at this point to add information from the study of lethal factors in *Drosophila* and the work that has been done particularly by Ernst Hadorn and his group. Here a similar type of study was made with an organism that has been studied to a great extent genetically but whose embryology and development have been less feasible to experimental studies as compared to some of the other forms. Yet the approach using specific lethal mutants has helped greatly to elucidate some of the problems in the development of the insect.

I think that, in the insect where there are distinct stages determined by the molting process, the very fact that genes cause lethal effects in specific stages is signifi-

[1] B. Mintz, The Institute for Cancer Research, Philadelphia.
[2] A. G. Searle, Radiobiological Research Unit, Harwell, Berkshire, England.
[3] F. Seto, Berea College.

cant. Further study along this line is one way of approaching certain problems of development.

You mentioned the antigenic approach. Hadorn has used a biochemical one in which he analyzed the amino acid contents of specific lethal mutants. The result in itself is not significant until it can be related to the particular morphogenetic changes or abnormality. I believe he obtained some very significant results using this approach. So your suggestion to apply an antigenic approach as a tool, I think is a practical one and would be another desirable way of approaching the general problem. Any information obtained by this means, I am sure, is going to be helpful in the total picture of gene action in development.

M. GREEN[4]: Dr. Grüneberg, do you think there is much that can be done experimentally with the mouse embryo? There are genes such as luxate that are known to affect the limbs and also the axial skeleton. It would be interesting to find out what the connection is between these two effects. Finding antigenic differences at an early stage might not give information about how the gene causes these two different effects. It seems to me that an experimental approach in this case would be useful. Do you think anything of this kind would be profitable?

GRÜNEBERG: There are, of course, many instances where the retrograde analysis has failed to identify a common morphological cause for the pleiotropic ramifications of a syndrome. You mentioned the effects of luxate on the axial and appendicular skeleton. Another typical case is fidget as described by Truslove ('56), where it is fairly obvious that the labyrinthine effects cannot be the cause of the ocular ones and vice versa. In such instances it may well be found that the common factor is a specific protein that enters into the development of more than one structure. However, it is easy to speculate, but difficult to prove such a relationship experimentally; one could hardly expect to achieve this in the initial phases of a research program. Pending success by immunological techniques, I would certainly regard it as a mistake to neglect other possible lines of approach.

DUNN[5]: I would like to raise a questi about what I thought I detected as *argumentum ad homunculum*. As I g the drift of the argument, the farther ba we go, the less likely we are to be able dissect individual processes. We wou end up by having all processes bound together in the fertilized egg. Was th the intention of the speaker, to make th implication?

GRÜNEBERG: No. I do not think that these antigens will be present *ab initio*, b that they arise epigenetically one after t other. If you can generalize from the sults of Konyukhov, proteins will tend make their appearance as the embryon process approaches, which for its comp tion requires the presence of these p teins. If so, we may not have to go ba much beyond the onset of gene effects identified morphologically—if indeed it true that the retrograde morphologic analysis has very nearly reached the lim of what it can do.

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[4] M. Green, Roscoe B. Jackson Memorial La ratory.

[5] L. C. Dunn, Columbia University.

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Genetics in Relation to Reproductive Physiology in Mammals[1]

W. F. HOLLANDER
Genetics Department, Iowa State University, Ames

In our ordinary Mendelian studies with mammals the importance of reproductive physiology impresses us most forcibly when something goes wrong, or when we switch from one species to another. When we graduate to study of reproductive physiology itself, we find that the situation is likely not to be "ordinary Mendelian" any more. We sense an aura of importance here not encountered with blue eyes or curly hair—this is in the medical or veterinary realm. Perhaps what we discover about sterility in mice can be applied to man. And much of the research in genetics of livestock is directed to the elimination of breeding difficulties.

With or without the aura, problems in these fields are of intrinsic biological interest. In this paper, I propose to give a cursory survey of these problems and new developments in their solution. In so far as possible the most recent source references will be cited.

The physiology of reproduction depends on a complex orchestration in which almost all the organs of the body play a part. Elucidation of the integration has been largely accomplished in experiments involving surgical disturbance (usually ectomy), injections of organ extracts, and grafting. Many textbooks and special treatises are devoted to setting forth the accumulated knowledge not only for the human but also various other mammalian species. Perusal of these works yields discouragingly little evidence of genetic studies. Interdisciplinary research is rapidly increasing, however, and it is generally recognized now that, underlying all physiology, is a not-immutable genetic foundation.

EVOLUTIONARY ASPECTS

Despite more or less comparability in anatomical structures concerned with reproductive physiology, mammals exhibit fantastic differences of functional detail. Even Asdell ('46), who was searching for patterns, uses the word "bewildering" on occasion. Young opossums look deceivingly like rats, but the reproduction of opossums differs from that of rats more than that of Reptilia from Aves. A sampling of species diversity, partly from Asdell, will point up the genetic aspects.

Ovulation in most species is spontaneous, but in some it is triggered by copulation. Thus estrus cycles typify rats but not rabbits. Timing of heat is varied; in some species it occurs seasonally, in others every few days, weeks, or months. There may be, as in mice, a postpartum heat. Heat usually coincides roughly with ovulation but, in bats of the temperate zone, may precede it by months. Special scents signal heat to the male in most species, but the rhesus macaque displays a perineal blush.

The adult testes in most species descend from the body cavity into obvious peritoneal outpocketings, which function to keep the testes cool. Whales, elephants, and bats violate this rule. In the deer family the males may grow enormous antlers annually, or in other cases have horns that grow throughout life. Polygamous species commonly show striking sex difference in body size, and the males enjoy mortal combat. By contrast, several male dogs may amicably pursue one bitch.

Coitus may be momentary, as in cattle, but up to 3 hours in the ferret. Remarkable differences of penis structure, shape, and excrescences are found, e.g., straight in cattle, bifurcated in opossums, screw-

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form in swine, and spiny in cats. The components of semen, such as choline derivatives and fructose, differ characteristically. In the mouse only one copulation ordinarily occurs because the semen coagulates in the vagina to form a rigid plug. In many other species repeated matings are common, so that the young in a litter are likely to be half-sibs. The vaginal plug in the mouse is not merely an exclusion device, but is necessary for development of corpora lutea (Lipkow, '59). Therefore, artificial insemination of mice requires a vasectomized buck as an adjunct. Pseudopregnancy from sterile copulation lasts about half the normal gestation period in mice and rats but up to the full period in the dog.

Corpora lutea, producing progesterone, seem always necessary for uterine development in successful pregnancy, but diversity occurs here too (Hisaw, '59). Usually one corpus luteum develops from each ovulated follicle, so that the number should correspond with the number of embryos. However, to produce a litter of four young a mink requires perhaps 20 ova, an armadillo only one. The mare ovulates successive crops of follicles in the early months of pregnancy, giving a number of corpora lutea. Parkes ('54) states that a pregnant elephant had 26 active corpora lutea.

Superfetation (fertilization of ova from successive ovulations for one pregnancy) is normal in mink but not in the horse. Implantation of blastocysts in most species is immediate but may be delayed for months in such species as seals, the roe deer, and armadillos, so that the actual gestation period in the latter situation is misleading. In mice there is some delay of implantation if the female is lactating.

In rabbits the blastocysts are large enough for the uterus to regulate their spacing before implantation (Böving, '59). Placentas are distinctive in gross form and histologic detail. At least in some mammals, including man, there is evidence that the placenta functions in part as a temporary endocrine organ, producing gonadotropic and estrogenic hormones.

Fetal development is very advanced at birth in the guinea pig but not in the rabbit. For parturition in many species relaxation of pelvic ligaments occurs (Hisa '59). For a few days, baby dolphins main tethered to the mother by the um lical cord. Mammary glands dif characteristically in structure, numb location, and milk chemistry. Especia in ruminants the colostrum is necessa to the newborn young as a source of an body globulins.

Maternal behavior is full of surpris The rabbit doe plucks breast fur to li her nest. Even herbivores like the c devour the placenta. The timid cat comes a dog-chasing fury.

Such a plethora of procreation pe liarities present fascinating riddles evolution. How rapidly, for examp could mammals progress from the mo treme to the marsupial stage? Or fr the marsupial to the insectivore? The steps, seemingly not great at first thoug become major on closer inspection, a abrupt transition becomes inconceivab Within these groups we find some of t possible substeps that might bridge t chasms, and these bolster the view tha multitude of minor changes were nec sary. Therefore, evolution was probab slow. Revolutionary changes probably gan as insignificant deviations.

Are there then general laws in the ph iology of reproduction? I doubt it, a echo the sentiments of Sir Solly Zuck man in his closing remarks at anot conference (Lloyd, '59), that generali tions may over-simplify. Even such seemingly obvious rule as that estroge hormone is produced by females is unre able—the testis of the stallion is a ri source. Another supposedly good princip that pituitary gonadotropin is required f maintenance of the testis, is also violate Smith ('44) showed that androgen wou suffice in the hypophysectomized monke

No matter what strange pathways species follows in this "Alice-in-End crineland" world, the normal outcome successful propagation. A function machinery nicely fitting reproduction in the animals' life activities has be achieved. Likely as not, then, the on underlying principle is that the end jus fies the means. Search for other gene laws, if conducive to ignoring adapti ramification, may be unrealistic. I the

fore favor the attitude of "vive la differ-ence!" which presumably coincides with the micawberism of Mother Nature's gene-ics.

OBSTACLES IN GENETIC ANALYSIS

Given two distinct types, the obvious ap-proach to genetical analysis of their dif-ferences is to cross. Crosses between genera or species, however, often yield more reproductive problems than solutions. Hybrid inviability has not been so well studied in mammals as in plants (Steb-bins, '58), where interactions between the hybrid embryo and the maternal parent are often the limiting factor.

A suggestion of such limitation is offered by reciprocal-cross differences with cattle × American bison: viable hybrids are rarely produced by the domestic cow, hy-dramnios usually being severe. Gray ('54) has excellently condensed the literature on mammalian hybridization; among labor-atory animals perhaps the most informa-tive and interesting results have come from crosses of *Cavia* species. In some of these the F_1 hybrids are so large that there is excessive natal mortality, but sterility oc-curs first in F_2 segregates.

Sterility of F_1 males is extremely com-mon in the tested mammalian species. It might be assumed that spermato-genesis is more sensitive to disruption by chromosomal pairing difficulties than is oogenesis; however, in bird hybrids, the reverse is found. At any rate, some hope of genetic analysis exists if any backcross-ing to parental species is possible (see Peters and Newbound, '57). Where both sexes of F_1 are fertile, as in subspecies hy-brids especially, the difficulties encountered are usually in description and classifica-tion of subsequent generations. Hybridiza-tion of laboratory mice with Chinese and Persian subspecies has given heterosis in reproduction.

Crosses among domestic breeds and varieties introduce other difficulties for genetic analysis. The species norm may not be available for reference, purity of parental forms may be questionable, and genotype-environmental interactions may be important. Segregations are often so confusing that analysis may be abandoned, and estimates of heritability substituted.

Part of such difficulties may be resolved with the aid of physiological study, to de-fine more clearly the nature of the "char-acters" being dealt with. For example, the character "heavy lactation" may be at-tributed to large udder size, or to high secretion of thyroxine or pituitary lacto-gen, for example. Turner *et al.* ('57) de-vised techniques for measuring levels of several hormones for individual lactating heifers and found consistent differences. Another example is the character *small litter size*, which might originate in lower ovarian or pituitary activity, or in em-bryonic mortality. Such increase in pre-cision of characterization is perhaps our best hope for future advance. There is however some danger in post hoc logic from inadequate studies. Such reasoning mars Stockard's work ('41) on the relation of bone and endocrine differences in breeds of dogs.

EXPLORING THE FIELD

It would be desirable to learn whether altered structure or function of a reproduc-tive organ is the immediate, direct effect of genetic difference, or a repercussion of an effect elsewhere. By ordinary theories and concepts in endocrinology, such alterations should be more or less channeled. The testis may serve as an example (fig. 1). Its range of pathology (Sohval, '56) is impressive. Who knows why a vitamin-E deficiency that sterilizes rats is not harm-ful to mice?

A now famous example of supposedly direct genetic effect is gonadal hypoplasia in Swedish Highland cattle (Koch *et al.*, '57; Venge, '59). When both gonads are affected, the animal is sterile, but nearly 90% of the cases involve only the left testis, and such bulls may show even above-ordinary potency. Eriksson's analyses (see reviews just cited) indicated the basis to be a single gene, recessive with incomplete penetrance, and affecting the ovary as well as the testis. The apparent gene frequency in the breed, 0.7, could be accounted for by breeders' selection of the whitest coat color, with which the hypoplasia is associ-ated (Lagerlöf, '56).

Certain other genetic changes affecting the testis will be considered in a later sec-tion. The literature on male reproductive

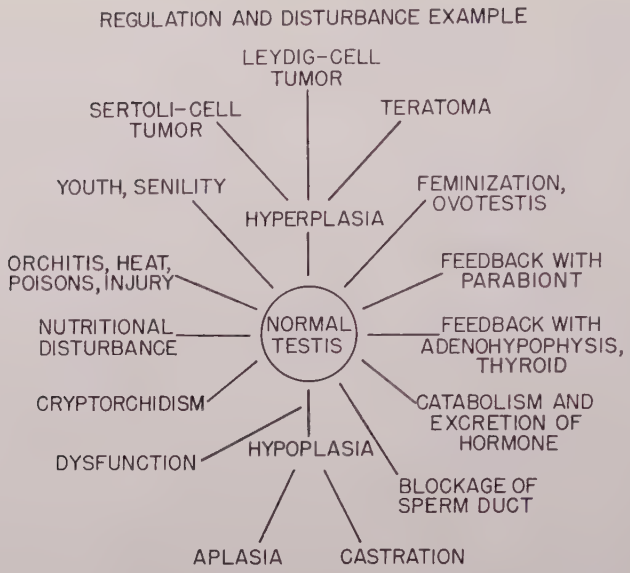

Fig. 1 A diagram to illustrate possible alterations of structure and function of an organ involved in reproductive physiology. Connecting lines suggest sites for possible genetic control or change.

disturbances is enormous, especially for man and livestock; useful additional reviews include Asdell ('58), Calisti ('56), Lörtscher ('58), Mixner ('59), and Rollinson ('55).

Female reproductive disturbances in livestock are responsible for immense economic waste. One of the common afflictions of dairy cows is cystic ovarian disease, signaled by nymphomania and often virilism. Since it is not a problem in beef breeds, a genetic basis might be reasonably suspected, and Henricson ('56) has adduced evidence for it. Other investigators minimize this explanation and search for endocrine causes. Since cystic effects can be induced by hormone disturbance artificially (Dawson, '57), possibly the estrogens of forage crops are a factor. These can interfere with breeding and even cause sterility in sheep (Engle *et al.*, '57). Natural estrogens in feedstuffs differ in their effects and by species (Magee and Matrone, '58). The recent advent of diethylstilbestrol in feeds has caused serious breeding losses in laboratory rodents (Wright and Seibold, '58), but here too strains differ in sensitivity. Drasher ('55) reported differences in uterine response.

Because of the possibility of inducing superovulation by means of gonadotropic pituitary hormones, we might deduce tha litter size (or twinning in monotocou species) is not primarily decided in th ovary. It has been demonstrated, hov ever, that ovarian sensitivity does diff in rats and can be selectively bred fo (Chung and Chapman, '58). The genetic of "maternal performance" (Bateman, '54 is extremely difficult to untangle. Falcone ('55) found a paradoxical effect in mic females from large litters tended to r produce poorly, apparently because of the less favorable nutrition while young. A kinson and Dickie ('58) have traced th "hyper-ovarian syndrome" of unmated I mice from DBA × CE strains to pituitar control. Selection away from the specie norm for one such character readily lead to unplanned consequences, and over-a reproductive efficiency may suffer (se Erb and Morrison, '59). Richter ('54 states that the rat in the course of domesti cation has developed increased reproduc tive capacity, apparently the result of in creased pituitary size and reduced adrena size.

Probably the best-analyzed example o gene-controlled endocrine sterility in mam mals is the pituitary-dwarf mouse. Th primary effect of the *dw* gene is aplasia o the acidophile cells of the hypophysis, wit

onsequent myxedema (Carsner and Rennels, '60; Wegelius, '59; Wykes *et al.*, '58). *ygmy*, another dwarf mutant type, is entirely different (King, '55).

The obese mutant in the mouse also eems to involve pituitary deficiency, but, this case, of gonadotropic hormones Jones and Harrison, '58; Lane, '59; Smithrg and Runner, '57). Control of hyperlycemia by strict diet may induce some reeding. Bielschowsky and Bielschowsky 56) described a similar mutant but one iat was less inclined to sterility.

Some out-of-the-way phenomena with hich I have had some experience may be etter understood by using genetic tools. or example, fusion of placentas in mice as readily distinguishable from identical winning by genetic differences in the emryos. The cause of the fusions was traced rgely to chance juxtaposition and could e increased by unilateral ovariectomy Hollander and Strong, '50) or other means crowding the sites (McLaren and Iichie, '59). In the detection of superetation, the use of genetically different ales in succession is desirable (Holnder, '59), to distinguish from delayed nplantation.

There has been a new surge of interest neural control mechanisms in reproducve physiology. Why do estrus cycles beome abnormal when female mice are aged in large groups? Apparently hypoalamic disturbance of pituitary function involved (Whitten, '59; Christian, '59). arly pregnancy may even be interrupted the proximity of a strange male, especially if wild-type (Bruce, '59).

Evidence indicates that gestation periods at least a number of species depend on e genotype of the fetus. The mule is a od example (Gray, '54). In cattle, prolnged gestation has been attributed to enetically abnormal fetuses (Koch *et al.*, 7). One type, in Guernseys, is characteried by aplastic pituitary gland (Kennedy *al.*, '57), and another in Ayrshires, by mply continuing to grow (Wilson and oung, '58). The evidence is good that mple recessive genes are responsible, but ow they fail to trigger parturition is unnown.

Uterine and vaginal obstruction in cat, termed "white-heifer disease," is a problem in Shorthorns. Apparently the famous Duchess line of this breed became extinct from this type of sterility. Rendel ('52), analyzing extensive data from Dairy Shorthorns, concluded that genetic linkage is not the explanation, but rather that the *roan* factor favors the trouble. There was indication, however, of fluctuating incidence by years also; possibly here again forage estrogens play a role, during embryonic stages (see Witschi, '59). Nalbandov ('58) notes that, in American swine, a common cause of sterility is obstruction of the oviducts, with hydrosalpinx. In mice, imperforate vagina occurs sporadically in certain strains (Grüneberg, '52); in females having the gene for Loop-tail (*Lp*), Strong and Hollander ('49) observed a far higher incidence.

Hermaphroditic and intersexual phenomena merit passing comment in this review. Perhaps the most remarkable genetic types known in man are the Klinefelter syndrome (females resembling males) and testicular feminization (males resembling females). Cytological methods of sexing have greatly aided in diagnosis (Nelson, '57; Marburger and Nelson, '57). In the last couple of years detailed mitotic chromosome analyses have been taken up; the Klinefelter syndrome is now interpreted as a trisomic (XXY) condition (Ford *et al.*, '59), and it has even been claimed that a sterile female type known as Turner's syndrome is XO (see Puck *et al.*, '60). With congenital adrenal tumors, precocious sexuality and apparent sex reversal are typical, and a genetic origin may be involved (Gurtner, '55).

Intersexuality in Yorkshire swine, studied by Johnston et al. ('58) apparently has a hereditary basis. Intersexuality in goats is limited almost entirely to genetic females homozygous for the dominant polled factor. Kondo ('55) concluded, in agreement with others, that two closely linked loci are involved. It seems to me, however, extremely unlikely that two separate mutations would have taken place so close together on the same chromatid.

Bradbury and Bunge ('58) described three hermaphroditic rats (oocytes in testes) of the Sprague-Dawley strain. Gynandromorphs have been found many times in the Bagg albino strain of mice (Hol-

lander *et al.*, '56). Use of genetic markers in trying to elucidate the mechanism of their formation is in progress.

Before closing this general survey, it is important to note ramifications of the physiology of reproduction and genetics into the field of cancer study. Strain differences and sex differences are an old story, but with some new versions. Prolonged endocrine imbalance is a powerful tool in creating specific types of tumors, given the proper genotype (Clifton, '59). For example, continued dosage of male mice of strain A with estrogen results in testicular change and eventually Leydig- cell tumors. F_1 hybrids are similarly sensitive; by means of testis grafts from sensitive and resistant parent strains, the difference was found to reside in the testis. Similar studies have demonstrated susceptibility to vaginal tumor to be local. And pituitary tumors are induced in strain C57BL mice by estrogen. Gardner ('58) found that backcrosses of sensitive F_1 hybrids to resistant parent type yielded a fairly definite 1:1 ratio.

The carcinogen methylcholanthrene when injected subcutaneously into early-pregnant mice of C3H strain ordinarily causes death of most of the embryos, The same dose injected into females of the JK strain had no evident effect (Strong and Hollander, '47). Sensitivity of mice to the tumor-inducing effect of methylcholanthrene has shown a paradoxical relation to litter sequence; young from late litters show greater sensitivity than from early litters (Markello, '58). Strong and Hollander ('52) found that in a strain of mice spontaneously developing tumors in the glandular stomach, the incidence was consistently and markedly low in breeder females compared to virgins and males. The physiological bases of these phenomena are not understood.

PLEIOTROPIC STERILITY

In the Ames laboratory we obtained, in a specific-locus X-ray experiment with mice, a new allele of p (pink-eyed-dilute) that proved to be homozygous male-sterile (Hollander *et al.*, '60). It has been named "p-sterile" and given the symbol p^s. There are other pleiotropic effects also: small size, nervous behavior, tooth-wear anomalies, and early senility. All these effec are recessive to both p and the wild-ty allele.

Female p^s/p^s mice breed to some exte but have rarely taken care of the young, that fostering is necessary. Investigati of the sterility feature of p^s/p^s mal showed two faults: poor libido and high abnormal sperms. Two males in about tested were briefly able to sire one or t small litters. Spermatogenesis is norm up to spermatid stages, when abnormali of the acrosome cap appears. Often t acrosome fails to form, although the tail normal. Sperm heads are extremely vari in shape and structure, even filamento Debris in the semen suggests that the n cleus often ruptures. The older a smaller males appear to have the mo abnormal sperms, though motility is n lost.

We attribute the sperm-head defec here to the acrosome failure, and that turn to the Golgi body. Since heterozygo males show normal segregation, and obvious proportion of abnormal sperm there is no support to the idea of dire control over the sperm's morphology by gene content.

There is no final evidence of course as whether p^s is a true point mutation, b linkage tests and viability indicate that cannot be a significant gene deficien The possibility that it is a pseudoallele p seems unlikely because the pigme phenotypes are identical. Assuming th p^s is a point mutation, what can we s about the gene action? I do not see h such varied effects on pigment, growth a teeth, behavior, and spermiogenesis together physiologically, or trace to so more fundamental derangement.

Apparently, p^s does not stand alone its pleiotropism. A ruby-eye mutant type the hamster is reported to become mal sterile after the age of about three month (Bruce, '58; Robinson, '58). Also, Jakw and Young ('58) describe subfertility a sterility in male red-eyed guinea pigs, wi failure of spermiogenesis. Male-sterility a pale-gray mutant in *Mastomys* appa ently also rests on absence of spermat stages (Menzies, '57). In the hou mouse, sterility effects accompany oth genes, notably the color mutants W^v a

a when homozygous, and alleles at the locus (Grüneberg, '52). In the mink, hackelford and Moore ('54) note that omozygous Stewart is male-sterile and ractically white. The probability of pleioropism in cattle has been mentioned; onadal hypoplasia with white, and white-eifer disease with roan. Intersexuality nd hornlessness in goats may yet prove o be another example.

Probably we should not be surprised t such syndromes, since experience with *Drosophila* provides plenty of analogous ituations. But for mammals these situaions present a challenge to common conepts, both in physiology and genetics, and heir analysis should be "good medicine." Also, much greater sampling of other loci nd other species seems needed; perhaps he confusion will be lessened by more lata.

INTERACTION OF MOTHER AND FETUS

In the course of linkage tests for the mutant hair-loss (*hl*) in the mouse we were impressed by some very bad segregation ratios. Nothing was amiss when the sire was *hl/hl* and the dam normal (+/*hl*). but in the reciprocal test crosses, there was a marked deficiency of +/*hl* progeny at the age of 5 or 6 weeks, when classification was made. Selective mortality of this class was demonstrated by several lines of evidence: (1) mortality before weaning age was much higher where the mother was *hl/hl* than in the reciprocal cross; (2) closely linked marker genes (on chromosome 6) showed similar ratio distortion when in this cross; and (3) many of the surviving +/*hl* had been runty in contrast to the good growth of *hl/hl* sibs.

Most of the mortality occurred during the first 2 weeks; many died at birth, or

Fig. 2 Alizarin-stained skeletons of mice about 1 week old, dead as a result of maternal antagonism involving the hair-loss gene. Note the many sites of fracture in the ossifying regions.

failed to inflate the lungs well and died cyanotic the first day. As growth went on, the litter could be rather easily separated into a thrifty and a poor class. The poorly growing young commonly had broken legs when about a week old. Alizarin-stained specimens show many fractures, including the ribs. If the young survived, however, the breaks healed, and after the age of about 2 weeks, growth became nearly normal. Stunting only occasionally persisted to maturity.

To test the possibility that the milk of *hl/hl* mothers disagreed with the *+/hl* young, progeny were transferred immediately after birth to normal foster mothers. The mortality and broken bones still occurred. A prenatal antagonism must therefore be involved (Hollander and Gowen, '59).

In searching through other variables, we found further that the mortality was greatly intensified where the dam had been nursing a previous litter while pregnant. Otherwise no increase of mortality with successive pregnancies was found.

To a certain extent the antagonism phenomenon here is similar to the effect of Rh antagonism in man. Therefore, blood of dying young was examined. No erythroblastosis and no icterus were found. R. D. Owen has confirmed this finding. It seems then, that a novel kind of incompatibility is involved.

For successful breeding, the worst kind of mating should be *hl/hl* female to homozygous normal male. Experience corroborates this expectation—practically all the young die or suffer broken bones or initial stunting.

One new fact has recently appeared that clouds the picture. Inter se matings of *N Ca hl bt/+ +hl bt* mice, which are moderately vigorous themselves and produce normal-sized litters, yield distorted ratios. Mortality of the young is high, but apparently without bone fractures, and the survivors are chiefly *+ + hl bt/+ + hl bt*. Matings of *++ hl bt/++ hl bt* ♂♂ with *N Ca hl bt/++ hl bt* ♀♀ have not shown such distortion of ratios, but the reciprocal mating has not yet given data.

A literature search has revealed no comparable antagonism phenomenon in mammals, except possibly in species hybrids. Interspecific transfer of ova demonstrat incompatibility effects in the uterus, ho ever, (Briones and Beatty, '54). Som thing of the latter sort seems also involv in the early death of yellow mouse hom zygotes (Grüneberg, '52).

Bateman ('54) by means of fosteri discovered an antenatal effect on grow of mice to 12 days postpartum, but n revealed by birth weights; "an intra-ute ine effect which is delayed in its acti until the postnatal period." The nature this influence was not discovered; it do seem similar to the *hl* antagonism effe though on a far less-drastic level.

Prenatal blood-type antagonism h been reported for rabbits (see Sawin, '55 Mitchison ('52) was unable to detect re cell antigens in mice until a week aft birth; he considers the mouse at birth co parable in development to the human fet of 3 months. Brambell *et al.* ('58), ho ever, found that hemolytic sera fed suckling mice can kill them.

Tolerance and interaction of mother a fetus are necessary developments in t evolution of mammals, and it seems pro able that genetic changes leading to di cord would be eliminated or forced in isolation. To analyze the adaptations, w may find the aberrations most informativ and this is particularly true in regard t production of congenital defects by m ternal influences. Disturbance of the ca bohydrate metabolism is one effective too hyperglycemia of the dam and excess a renal corticoids may adversely affect th embryo (Hoet, '54; Kalter and Warkan '59). Maternal effects on the expression the Fused gene (*Fu*) in the mouse (Grün berg, '52) may be of such origin.

Returning to the case of antagonis with *hl*, we might inquire whether hormon disturbance might be a factor. The pheno type of *hl* suggests a relation to matur tion of the gonads, and it has been know for a long time that sex hormones hav curious relations to hair growth and bald ness. Estrogens tend to inhibit hair regen eration in rats and other animals (Johr son, '58). I have castrated a few mal *hl/hl* mice 4–6 weeks old (adolescence) an observed arrest in the loss of hair, particu larly in those youngest at castratio Whether the arrest will be permane

eds further study. At any rate, no direct nnection of hormones to the antagonism seen as yet.

Assuming that there is some unique operty of the *hl* mice to account for the ıtagonism effect, we should investigate imic mutants. Do the very similar phenopes involving other loci, such as hairless, id in other species, such as deer mice and ts, also show antagonism? I know of no idence.

CONCLUSIONS

I make no claim here to completeness, derliness, or great theoretical advances. ather, emphasis is laid on diversity, hapzardness, and the unexplained correlaons. Study in this field is likely to be endss, and mainly devoted to dissection of nomes, species by species. In such analis a closer collaboration of physiologists d geneticists is a desideratum. Just as netic tools have been revolutionizing icrobiology, they are beginning to gain verage in endocrinology.

In relation to practical interests, what n we say has been gained so far? Perps only a greater appreciation of comexity, or sense of caution in breeding lection; pulling a string here may unvel the physiological fabric elsewhere. n the other hand, perhaps we will be ore reluctant to liquidate defective types, nce they may be our chief means of getng greater insight.

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I am grateful to Dr. Robert M. Melampy r various suggestions and discussion nd to Miss Joan Sturtevant who prepared e alizarin-stained specimens.

OPEN DISCUSSION

AUSTIN[2]: I would like to comment on a oint that Dr. Hollander made in connecon with the blockage of pregnancy in ice by proximity with strange males. his is a finding by Miss H. M. Bruce. She as published a preliminary report in NAURE and the full report appears in the sue of THE JOURNAL OF REPRODUCTION ND FERTILITY that has just appeared. Bruce observed that if mice were mated the normal way and the male was reoved on the day of plug and a strange male—preferably a dark-eyed male—was allowed to come into proximity with the female, but not into physical contact, the pregnancy was blocked in 80% of the females. This was apparently attributable, not to an interference with embryonic development as such—which began in a normal fashion, but to the uterus returning to the estrous state. If allowed, the female would mate with the strange dark-eyed male and become pregnant to him.

FRACCARO[3]: I want to correct a slight misstatement that was made in defining patients with Klinefelter's syndrome as "females resembling males." There is no reason to call them females: they are intersexes with an abnormal sex chromosome constitution of the XXY type.

HOLLANDER: I am sorry if I over-simplified or misstated it.

OWEN[4]: I would like to comment briefly on some studies of the hair-loss interaction, conducted by Dr. Andree Dubert in my laboratory this past year, with stocks kindly provided us by Dr. Hollander. At first this looks like an ordinary maternal-fetal incompatibility in which a maternal immune reaction is directed against some product of the normal allele in the heterozygous young. But we looked in vain for any kind of sensitization of the mother. We could not detect serum antibody with any of the techniques available to us, including cytotoxic tests on normal nucleated cells. We did some skin grafts to post-partum hair-loss females whose normal young had shown the apparent incompatibility, but found no indication of tissue sensitization of the females. Dr. Dubert did a little work with radioactive strontium injected into these animals. Although the results are inconclusive, there is some evidence that hair-loss mice maintain and perhaps require a considerably smaller pool of calcium than do mice heterozygous or homozygous for the normal allele. Dr. Dubert suggested a model in which the hair-loss females with this smaller pool have too little to contribute to the normal young, but enough for their hair-loss

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[2] C. R. Austin, National Institute for Medical Research, London.

[3] M. Fraccaro, University of Uppsala, Sweden.

[4] R. D. Owen, California Institute of Technology.

young, whereas in the reciprocal mating the normal females have a large enough pool so that all three genotypes progress satisfactorily. I should emphasize again that the data are not fully persuasive in this regard, and I would not wish to suggest a primary relation between the calcium pool and the growth abberrations of the affected young. I only mention that an apparent incompatibility need not have an immunogenetic basis; it may be, for example, nutritional in nature.

HOLLANDER: I hope that people who are working with hairless mice, also the deer mouse and the rat, will try to make matings that will be parallel to mine with the hair-loss condition and see whether the same sort of antagonism will happen there.

So far, females of the hairless types have usually been just considered a waste and not of use in breeding. I hope they will be tried more carefully in the future.

BRADEN[5]: We in Australia have been very concerned about estrogens in pasture. The evidence is that, although sheep may be very much affected, cattle are not.

HOLLANDER: Which breed of cattle?

BRADEN: Beef breeds. Castrated male sheep are very sensitive to the effects of estrogen; they lactate, and the bulbourethral glands become greatly enlarged, causing death from uremia. But the steer grazing on the same pasture is not affected at all.

The second point—in relation to the slide showing abnormalities of sperm from your p^s mutant, the types are very similar to what has been found by Bryson, for instance, in 1944 in males heterozygous for two *t* alleles.

The third point is just to clarify what I have said about genes having an effect on sperm morphology in rabbits. The work was that of Beatty in Edinburgh. The males were not sterile. The difference was in reaction to DOPA.

There is no evidence to implicate pasture estrogens in bovine infertility.

HOLLANDER: I just threw the estrogen idea in for stimulus in cattle. I have nothing to do with cattle myself, and I could only make a guess. I hope it has been of value as a stimulant.

In regard to the sperm abnormalities in p^s males, I think that the exploded type of head, where there is a mass of threads practically nothing, is not found in the types. Rajasekarasetty showed diagra of various kinds of abnormalities that we found, and that is not one of them. Fro all the debris we see in the field, Dr. Bry and I, who have studied this, have abo concluded that the acrosome fails to i clude the nucleus properly, and it m rupture and release chromosomal mater which then becomes chemically chang so it is no longer responsive to Feulgen.

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urrent Status of Mammalian Immunogenetics

RAY D. OWEN
California Institute of Technology, Pasadena, California

During this century increasing use has en made of the techniques of immunol- y for the recognition and study of in- rited individual differences. The field is become broad and diverse; currently, search is particularly active in the areas genetic control of cellular and soluble itigens, tissue transplant incompatibility, production and maternal–fetal interac- ons, and in the aspect of somatic cell netics that deals with antibody forma- on. Only a selection from these currently tive areas will be attempted here; this lection will not pretend even to give oper priority recognition, but will aim illustrate what is going on in the field. veral relatively recent reviews (e.g., wen, '58, '59; Stormont, '58; Irwin and one, '60) provide guides to earlier litera- re.

CELLULAR ANTIGENS

Tests with antibody reagents frequently veal inherited individual differences in e specific reactivities of red blood cells. number of independent genetic loci are pically concerned with this characteris- , but as a rule with very few exceptions e effect of any given allele is recogniz- le in any residual genotype or environ- ent. There have been few evidences of ther allelic or genic interaction leading the suppression or distortion beyond cognition of the effects of genes control- ng the red cell antigenic characteristics. his situation led early to the assumption at the cellular antigens must be virtually mediate products of the genes that con- l them. In spite of growing lists of amples of interaction this assumption ll seems generally valid, but we must cognize that the term "antigen" as used this context has a restricted meaning. e specificities toward which antibodies e directed are small areas on the sur- ces of large molecules. Saying that a particular specificity is directly related to the allele that controls it does not rule out the possibility that numerous genes may be concerned with the formation of the ultimate macromolecule. In fact, macromolecular antigens that have been studied in chemical detail, the soluble human blood group substances, are affected by several independent loci (see Morgan, '60). Similar genic interactions apply to soluble substances of sheep plasma, which adsorb to surfaces of red cells and became secondary antigenic properties of the red cells (Rendel, '57).

Typically in red cell immunogenetics, reagents are derived by antibody absorptions to recognize "unit specificities," and each distinctive reagent is assigned a symbol, such as anti-A, to record that specificity. A unit reagent is defined by the ability of each reactive cell to absorb all of the antibody capable of reacting with any other positive cell; negative cells remove none of the relevant antibody. Given a sufficiently large battery of reagents for individual differences in the red cells of any species, it generally turns out that particular genes acquire complex designations in terms of this symbolism. For example, an allele at the B locus in cattle may be represented as $BGKO_xY_1A'E'_3$ *I'6,7, 8*, or in the *H-2* complex in mice as *ACEHKY*. The significance of this complexity of symbolization is still debatable. There can be little doubt that there is structural differentiation within a gene. Specific differences in gene products or effects, of the dimensions recognized by antibody molecules, can be expected to combine the effects on a macromolecular antigen of different areas within a gene. The diversity of alleles present at particular loci may well arise, at least in part, from mutations at different subgenic sites and from events like gene conversion dur-

ing DNA replication in heterozygotes. Closely linked but separately particulate genes may also play a part in this diversity; in fact, some possible recombinational events of pseudoallelic type have been reported, in the mouse by Allen ('55), Amos *et al.* ('55), Hoecker ('58), and Gorer and Mikulska ('59); in the chicken by Scheinberg ('56) and Briles ('58); and in cattle by Stormont ('55), Rendel ('58), and Datta *et al.* ('59). Stormont (personal communication), who first identified mutation-like changes at the B locus in cattle, has observed a total of five such changes and estimates the frequency of exceptional events at this locus as about 10^{-3}. Whether the variant antigens in these reports are in fact the result of mutation, recombination, or gene conversion, or may even represent some such phenomenon as paramutation (see references in Brink, '59) cannot be discerned by the available data. In the absence of evidence to the contrary, it is probably best to accept them as recombinational in origin. Snell ('58) favors accepting the term "locus" for the *H-2* complex in mice, even for a region in which apparent recombination may occur, until a unit with some quite different effect is mapped within the region, separating it into two or more clearly discrete elements.

Immunochemical considerations emphasize that there are clear limitations on the specificity of antibody reactions; a shared symbol in the formulas of two or more antigens does not necessarily mean that these antigens are identical in any detail of their structure. Batteries of antibody reagents for sets of related antigens would be expected to produce a maze of symbolic complexity in the representation of the reactions of these antigens, even if they differed at a single specific site under the control of a series of true, point-mutational alleles. This point need not be belabored here; it has been discussed elsewhere (Stormont, '55; Owen, '58). Only a genetic analysis of the subdivisibility of the apparent complexes, upon which a start has been made, can tell us in time the extent to which the symbolic complexity of the cellular antigens relates in a one-to-one way to the complexity of the genes and their products. And only a detailed immunochemical analysis, u which a start has hardly been made, tell us the extent to which the symb complexity of the antigens relates to tails of discrete specificity in their st ture corresponding to distinct compone of heterogeneous antibody populations

Deviations from a simple and straig forward relation between the genic subgenic) symbolism and the reality serological reactions have become incre ingly evident of late. A sample of th deviations includes three categories:

Intra-allelic. In the DCE symboli for Rh, reagents for $\overline{ce}$ came to attent some years ago. These reagents react o to cells in which *c* and *e* are present gether in the allelic formula; they w assigned the symbol anti-**f** (Sanger *et* '53). The reactions do not depend anti-**c** or anti-**e** as separable antibody po lations. Somewhat similarly, reage anti-$\overline{CD}$ have been known for some tir these antibodies are absorbed by most c having either *C* or *D* in their symbo genotype and have been assigned the sy bol anti-**G** (Allen and Tippett, '58). Ros field ('58) suggests that **D** and **C** shar common substrate material and that G part of that substrate. Rosenfield a Haber ('58) have reported a brilli study of anti-$\overline{Ce}$. It is conceivable that greater reactivities of Ce bloods to par ular anti-C reagents previously descri as a "position effect" (Race *et al.*, '5 may in fact relate to this type of antibo rather than to a true position effect in genic control of the antigen. Such re tions suggest a specificity common to a gens that, on other grounds, seem to clearly distinct; they are similar to type of reaction long known in cat blood, through which for example "anti-K reagent" could as well be des nated "anti-BGK" (Stormont *et al.*, '5 because cells reacting to this reagent ways react also to anti-B and anti-G. such circumstances, the physical meani of the symbol **K**, or of **G** for $\overline{CD}$ or **f** $\overline{ce}$ is debatable. The extent of allelic versity in these instances is great a almost any new reagent will make n distinctions among alleles up to that po considered alike and throw old categori

eviously considered distinct, into com-
on groupings.

Interallelic. Perhaps the most interest-
g example is Cohen's work ('58) in the
bbit, where a new specificity is found
be characteristic of the heterozygote for
o particular alleles, and a third allele
noted that in the homozygous condition
nfers a reactivity similar to that found
ly in the heterozygote for the other two.
terallelic interaction to produce "hybrid"
ecificities was reported in birds by Bryan
d Miller ('53), and has been claimed
the *F-V* locus in cattle (Borel and
one, '59). Another type of interaction
s been reported for P^k in human red cells
ortekangas *et al.*, '59); here, P^k appears
be expressed in genotypes P^k/p and
$/P^k$ but not in P^k/P_1 or P^k/P_2. All P^k
dividuals have anti-**P** naturally present
their serum. Still another type, evident
the phenotypic level, is the partial sup-
ession of anti-$\mathbf{Rh_0}$ reaction noted when
e allele conferring the $\mathbf{Rh_0}$ reactivity is
ired with an r' or r^y allele (Ceppellini,
2; Chown and Lewis, '57). Another ex-
mple is the inclusion of both A and B
ecificities in the same precipitable mac-
molecule, the soluble blood group sub-
ance of *AB* heterozygotes (Morgan and
atkins, '56). Taken together, such evi-
nces of interallelic interaction suggest
at the gene-controlled specificity is at
ast sometimes incorporated in macro-
olecules assembled at some distance
om the gene.

Intergenic. Most of the evidence for
operation of genes at clearly separate
ci in elaboration of cellular antigens
ates in fact to soluble antigens secon-
rily acquired by red cell surfaces, or to
ner subjects that will be considered else-
here in this paper. The suppressor for
od group B on human red cells (Levine
al., '55) is one example of a rare excep-
n. The rarity of intergenic interactions
red cell specificities is provocative.
r other chemical effects on cells in gen-
al, such as particular amino acid re-
irements, numerous genes may have
e same essential end effect through
ocking any of several points in the bio-
nthetic chain leading to the formation
the substance. Simple substances
ould be active as haptens affecting the
specificities of cellular antigens, and we might therefore expect to find numerous genes affecting the same terminal unit specificity. That we do not suggests that *RBC* immunogenetics deals mainly with specificities acquired during the assembly of macromolecules. This line of thought provokes further speculation that we shall not indulge here. We should note again, however, that the critical groupings must still be small relative to the ultimate macromolecular antigen, which may take its final form, as in the soluble antigens, under the influence of several independent loci. Each critical grouping seems to relate uniquely to a particular locus, often subject to multiple allelic variation.

Before this brief section on the cellular antigens is concluded, some reference should be made to antigenic characteristics of cells other than *RBC*. Of course, some of the specificities of *RBC* are determined by genes affecting other tissues as well—e.g., the human blood groups and the *H-2* groups of mice. In the case of *H-2*, there are complications; for example, some mice seem to have present elsewhere in their bodies antigens of the *H-2* complex not detectable on their red cells (Amos *et al.*, '55). The ability of cells to take up antibody without giving evidence of reaction is noted in other systems as well; for example, cross-reacting eluates may be obtained from cells not containing the specific antigens with which these antibodies are supposed to react (Hubinont *et al.*, '59). In other parts of the field of immunology, particularly those dealing with hypersensitivity reactions, conspicuous differences in antibody effects depend on the ability of the antibody to fix to tissues. Sites on the antibody molecules distinct from those involved in reactions with foreign haptens are important in these reactions, and therefore techniques of *in vivo* absorption (Amos, '55) may sometimes give misleading indications of the tissue specificities of an organism. Differences even in RBC reactivity are common among strains of mice; for example, strain A RBC can often be tested in saline media, whereas many other strains require special techniques for demonstration of reaction with the same antibodies. The genetic basis for strain dif-

ferences of this kind has hardly been explored; it would be interesting to know whether they parallel differences in anaphylactic sensitizability (Rothberg and Talmage, '60). Ipsen ('59) has reported marked differences in immunizability among strains of mice.

Much inherited individuality is at hand for definition in the leukocytes, accessible through such test techniques as leukocyte agglutination or cytotoxic determinations of various sorts (see references in Rood *et al.*, '59 and Butler, '60). The importance of these antigens in maternal-fetal incompatibility is indicated in several studies (e.g., Hitzig and Gitzelmann, '59; Lalezari *et al.*, '60).

TRANSPLANTATION ANTIGENS

Evidence that rather large numbers of independent loci affect the histocompatibility characteristics of cells and tissues has been reviewed repeatedly (e.g., Owen, '59). Only a few points of current interest will be taken from extensive current literature. Until very recently only in the mouse had specific loci been identified with histocompatibility effects, largely through the efforts of Snell, Gorer and their colleagues (Snell, '58). A histocompatibility hemagglutinogen has now been identified in the rat (Bogden and Aptekman, '60). The prime importance of *H*-2 variation in the mouse has been demonstrated in several connections. In our laboratory (R. D. Owen, R. Barth, A. Dubert, and Z. Collins, unpublished), we found that segregating *H*-2 incompatibility contributed significantly to skin-graft survival times even against the background of numerous other histocompatibility differences among backcross progeny after a cross between two inbred lines. A sex difference between donor and recipient within *H*-2 classes also contributed discernibly to the rate of rejection of the transplant; males retained male skin transplants significantly longer than did females. The magnitude of this sex effect was surprising because, when sex is the only difference (e.g., within an inbred line), male skin transplants are sloughed only slowly by females (Eichwald *et al.*, '58). In our experiment, *H*-2 incompatibility and sex incompatibility seemed to interact essentially additively in the termination of median survival time. C among those recipients compatible both *H*-2 and sex were relatively long-t survivals noted; in this class a fair p ability of extended survival could be dicted for any given trial. Observati of this sort give hope to the possibility bringing histoincompatibility under s degree of control through matching m factors and ameliorating the effects minor differences.

At present it seems that strain varia in the sex histoincompatibility characte tic relates not to differences among chromosomes, but to differences in abilities of females of the different li to react to a common male antigen or of antigens (Billingham and Silvers, Bernstein *et al.*, '58; Eichwald *et al.*, Zaalberg, '59). There is no compell evidence that these antigens are associa directly with any specific locus in th chromosome; they may relate in less-di ways to the complex phenomena of differentiation controlled in part by c mosomes other than the Y. Other m complicated sexual effects have been i cated by the work of Barrett *et al.* ('5

In general, present evidence indica no interaction in genetic control of hi compatibility characteristics (Martin Shapiro, and Good, '59). Rejection of tain parental tumors by the F_1 at particu cell doses (Snell, '58) at first glance s gests a recessive specificity. But the rejections are not increased by preimmu zation, and seem therefore to represen physiologic interaction not directly munogenetic in origin. Fox ('58) sugge that genetic interactions will be found techniques of test become more delic and precise. Studies by Berrian McKhann ('60) give clear indication immunologic interactions. For exam in lines otherwise coisogenic, an *H*-3 ference does not immunize for accelera rejection of skin grafts when spleen c or extracts are injected intraperitonea If, however, there are differences at b *H*-2 and *H*-3, then immunization is acc plished for both. The further observat that skin transplants or epidermal suspensions, in contrast to spleen cells extracts, preimmunize for an *H*-3 dif

ıce alone suggests a form of tissue differentiation. Tissue-specific antigens imɔrtant in transplantation reactions are ly beginning to come under investigaɔn; antigens unique to particular tumors ay prove of medical significance (e.g., lber, '59). The failure of spleen cells to ımunize for accelerated skin graft reacɔns in some combinations seems at variıce with their ability to confer tolerance skin grafts in these combinations if they e injected into the neonatal animal. owever, the degree to which the speciity of immunologic tolerance parallels at of immunization remains debatable Terasaki *et al.*, '58; Hašek *et al.*, '59). our present genetic consideration, we ed only note that evaluation of the ature of immunogenetic control of transantation antigens will doubtless reflect part the characteristics of the system ed to test for these antigens.

Gene-controlled incompatibilities affectg the success of parabiosis in mice have veloped increased interest of late (Eichld *et al.*, '59). Major histocompatibility fferences frequently effect disjunction om parabiosis or death of one or both rabionts. But even major differences e sometimes tolerated (Pilgrim, '59). rabionts with few or minor differences, ch as particular parents and F_1 hybrids, ay regularly remain in successful union er long periods of time. Under these cirmstances, members of the pair may acire tolerance for each other's tissues reined after surgical disunion (Rubin, '59; artinez *et al.*, '60). Pairs of unlike sex, thin lines showing sex incompatibility, ay remain in compatible union over long riods. The female member of the pair, en as an adult, may acquire tolerance of ale tissues retained after surgical dision (Mariani *et al.*, '59). When a mouse an inbred strain, rendered tolerant to sue transplants from another strain by e neonatal injection of spleen cells, is aced in parabiosis with an untreated ember of its own strain, the latter frently acquires tolerance like his treated rtner's, passively transferred by way of e parabiotic vascular connection (Marez, Smith, *et al.*, '59). It has been sugsted (Rubin, '59) that what is really acired through parabiosis is a type of immunologic enhancement. Passive transfer of the enhanced state across the parabiotic junction could be explained in terms of Kaliss' interpretation ('58) of enhancement. Studies on the humoral antibody responses of disjoined parabionts would be of interest. The activity of globulins in inducing acquired tolerance to parabiotic union in rats (Kamrin, '58) should also be noted in this connection.

SOLUBLE ANTIGENS

I shall limit this discussion to γ-globulins of human and rabbit sera. Some years ago, Oudin ('56a, b) reported that, when he injected precipitates formed by the antibodies of particular rabbits into other rabbits, he obtained antisera capable of precipitating the γ-globulin of the donor rabbits. When antirabbit - globulin antisera obtained in this way were tested in an agar diffusion system against the normal sera of numerous rabbits, much inherent diversity became evident. At present (Oudin, '60), two independent genetic loci have been identified with this diversity. One includes a series of three alleles, and confers the specificities designated **a**, **f**, and **g**, respectively. The other, also a triple allelic series, confers the specificities **b**, **c**, and **d**. Thus a rabbit can have any one (homozygous) or any combination of two (heterozygous) specificities of the series **a**, **f**, **g**; and any one or combination of two of the **b**, **c**, **d** series. Similar observations, confirming and extending those of Oudin, have been reported by Dray and Young ('58, '60) and by Dubiski, Dudziak, and Skalba ('59) and Dubiski *et al.* ('59). Oudin ('60) reports that **a** and the **c** specificities are each found on two separable populations of serum protein molecules.

Primarily through the efforts of Porter ('59) but with confirmation and extensions by others (e.g., Nisonoff *et al.*, '60), the structure of the antibody molecule is being dissected. Enzymic digestion fractionates antibodies into three reasonably well-defined components; of these, I and II, each of molecular weight ~55,000 seem each to carry one of the two combining sites of the originally bivalent molecule. These two fractions are nearly identical in their amino acid composition but

are separable chromatographically. Fraction III, of molecular weight ~ 80,000, has no antibody valence site, but is effective as an antigen, and in fact has most of the species-specific antigenic property of the antibody.

The combination of structural and chemical characterization of the antibody molecule with the immunogenetic approach initiated by Oudin is enlarging our understanding of the nature of gene action in the control of protein specificities. Already it seems that genes at two loci interact in the formation of the ultimate macromolecule and that more than one class of macromolecules may be affected by the same allele. The antibody molecules of a serum are heterogeneous, however, and the current status of these studies would still permit confusion between mixtures of antibodies with individually separate specificities on the one hand, and individual molecules with two or more specificities per molecule on the other. This uncertainty is not insoluble, and publication of further studies can be anticipated with interest. One approach of value might be to apply the technique (Morgan and Watkins, '56) for definition of the AB substance to the antibodies as antigens. For example, if Oudin's specificities **a** and **f**, in an *a/f* heterozygote, are part of the same molecule, precipitation of a solution of the globulin with anti-**a** should also precipitate the f reactivity. Similarly, precipitation with anti-**a** of the γ-globulin of an *a b* rabbit should also remove *b* reactivity from solution if the alleles of these two genes affect the same individual antibody molecule.

Another approach to genetic control of γ-globulin specificities deals with the Gm characteristic of human globulin. This derives from Grubb's observation ('56) that certain sera from patients with rheumatoid arthritis (RA) agglutinate human red cells of group O that have been coated with particular "incomplete" Rh antibodies. Grubb and Laurell ('56) reported that a majority of normal human sera inhibit this agglutination; it was later found that the inhibitor was γ-globulin. The test system therefore involves determining whether a given human γ-globulin will combine with the antibody-like material found in RA and thus block this mate from agglutinating globulin-coated t cells. Persons whose sera accomplish t inhibition readily are designated Gm (a those whose sera are much less inhibit (the difference is reported to be at le tenfold) are described as Gm (a—). Th have been reports of intermediate typ and of the distinction of additional cle cut types, such as Gm^b, Gm^x, and Gm-l (see Steinberg, Giles and Stauffer, ' Steinberg, Stauffer, and Fudenberg, '6 Only a single locus has so far been ide fied with Gm variation, but much rema to be learned of the genetics of this ch acteristic. Many variables have to be c trolled; for example, Linnet-Jepsen ('5 described the basis for selecting the a Rh "incomplete" sera to be used for c ing test cells. Of 66 high-titer anti-R investigated, only 6 proved useful for c ing. Five of these were from Gm (a individuals. Eighty of 450 RA sera g reactions with the anti-Rh_0 coated red ce but the reactions were variable in streng and avidity. The RA sera must be select not only for their high titer in the aggl nation test, but also for failure to sho prozone and for their ability to be inhibit by pooled human γ-globulin. Only ab 10% of the RA sera with positive aggl nation reaction could be used in the agg tination inhibition test. Most of the use sera were from Gm (a—) donors. A markable test system for a similar sort variation involves the effect of a g etically determined human serum fac on a mating reaction in yeast (Steinb and Giles, '60). Current studies of m loma γ-globulins in relation to normal globulin antigens (Dray, '60) indicate t the myeloma protein corresponds, in dividual cases, to either but not both t normal 7S components identified rhesus monkey precipitins. The nor components may be produced by distin cell types; only one of these populatio may go wild in any particular case myeloma.

As in the rabbit γ-globulin types (s Brambell *et al.*, '60), the Gm groups of h man infants reflect the mother's rather th the infant's type. The Gm group of t human infant is not fully developed ev at 8 months postpartum (Linnet-Jeps

al., '58). Since this maternal serum pro- n persists for considerable periods in e infant's circulation, we might expect persistent tolerance toward antibodies of e maternal type, when they are tested antigens. It would be interesting to ow whether evidences of such tolerance n be detected in adults, either rabbit or man. An extension of studies of this rt of variation to the mouse would be of eat potential interest because of advan- ges of the mouse for genetic studies. onald S. Shreffler in our laboratory (un- blished results) has established the netic control of individual variation in rtain mouse serum proteins, but they e β- rather than γ-globulins.

ANTIBODY FORMATION

Much has been said about antibody for- ation in the 3 years since Schweet and '57) last ventured into the rough terrain this field as an aspect of somatic cell netics. At that time we were primarily ncerned with the suggestion that anti- dy formation must reflect an aspect of NA control of protein specificities, by ay of DNA effects on ribonucleoprotein mplates directly concerned with anti- dy synthesis. In the interval, emphasis s shifted. The implication of DNA dif- rences has been widely, though not uni- rsally, accepted, and the basis for the fference has become a major point at ue. Many immunologists retain the ore classical view that fragments of anti- n retained over long periods of time con- ue to affect the specific synthesis or tiary folding of globulin molecules rough a mechanism of direct template tion (e.g., Haurowitz *et al.*, '59). Others, rticularly those concerned with genetics, w incline toward Burnet's hypothesis 59) of clonal selection, popularized and tended by Lederberg ('59). Talmage 9) also prefers random mutational rather an antigen-directed origin for the cellu- mechanisms synthesizing antibodies, d calls attention to the increased infor- ation available to mixed systems through e varying proportions of heterogeneous tibodies commonly present in immune ra. Monod ('59) and others have ex- pited parallels with induced enzymes, d Schultz ('59) has considered the pos- sible relevancy of epigenetic effects on cell phenotypes. Bussard ('59) has published a perceptive review.

The main experimental developments in this area relate to production of antibodies by single cells isolated from sensitized individuals to microdrops in tissue culture. Here the findings of Cohn, Lennox, Horibata, and Attardi contrast sharply with those of Nossal and Lederberg, with regard to the frequency with which two types of antibody are produced by a single cell isolated from a doubly stimulated animal. Nossal ('60) continues to find no significant doubles, but Attardi *et al.* ('59) find them frequently. There is little doubt that single cells do indeed produce more than one type of antibody, and under the circumstances of this observation this would seem to render the clonal selection hypothesis in its simplest initial form tenable only with difficulty. We are still, however, some distance from any real understanding of the process of antibody formation. Recent work implicating genes in the control of specificity of antibodies as antigens, reviewed earlier in this paper, combined with indications that the whole process of antibody formation is accomplishable in the simplified situation of tissue culture (Fishman, '59) and Gitlin's current report ('60) of differences in the primary structure of related purified antibodies point to exciting times in this field in the near future. It is not entirely clear from his abstract whether Gitlin's observation may relate to differences in the composition of antibodies from individual rabbits, rather than to differences in the primary structure of antibodies as a function of their specificity.

IMMUNOGENETICS AND MAMMALIAN REPRODUCTION

Immunogenetic interactions of members of successive generations probably occur at all stages of mammalian development, from preliminaries to fertilization through the neonatal period. For a consideration of sperm characteristics and fertilization, see Braden (this symposium), and for a discussion of maternal-fetal incompatibility see Hollander (this symposium). Because of space limitations, and in view of the inclusion of related material elsewhere

in this symposium, no further discussion of these subjects will be undertaken here.

Immunogenetic aspects of the transmission of maternal globulins to the infant in both man and the rabbit were mentioned earlier in this paper (see also a comprehensive and excellent review by Brambell, '58). The transmission of γ-globulin across the placenta of the Rhesus monkey is selective; it occurs, according to Bangham *et al.* ('58), 15 to 20 times as easily as labeled albumin, and α_2- and β-globulins do not appear to reach the fetus at all. The endoderm of the yolk sac or the gut of other mammals transmits globulin selectively in a fashion similar to the primate placenta (see, however, Anderson, '59). In the rabbit it appears to be Porter's fraction III of the antibody that is primarily responsible for selective transmission (Brambell *et al.*, '60).

The existence of placental fusion in the mouse has again been called to attention (McLaren and Michie, '59); rejection of reciprocal skin grafts between the members of one pair of mice born with fused placentas suggests that there was no substantial vascular continuity in this case at least. The unique effects of parabiosis between adults discussed earlier raises again the question of the degree to which the maternal-fetal relationship may be analogous to parabiosis in its immunologic effects. It is true that no systematic continuity exists between maternal and the fetal circulations; nevertheless, intact cells can be transmitted in either direction across the placenta with some degree of frequency, and subcellular antigenic material must be exchanged between mother and fetus. The evasion of homograft reactions between the maternal and fetal parts of the placenta and between the embryo and the mother is still a riddle. Some studies of homotransplantation into or with the placenta are of interest in this connection (Wilson, '60; Payne and Payne, '60). If the maternal part of the placenta were a very effective site of immune response, it is conceivable that the escape of fetal antigens into the maternal circulation, or their residence or disposal there in such a way as to induce systemic immunity in the mother, might commonly be prevented, much as Rh-positive fetal material fails to sensitize an ABO incompatible mother (Levine, '58).

It would be nice to find that, under particular endocrine conditions of pregnancy and in the pseudoparabiotic relationship of fetus and mother, there a specific desensitization of the mother antigens being elaborated by the fet We could even conceive, in terms of clonal selection hypothesis, that pot tially reactive clones might be particul subject to destruction in the mother d ing pregnancy. Unfortunately, howe such direct evidence as we have does appear to support such speculation, least in the mouse (see Brent, '58, p. 29 On the other hand, there are some ind tions (Peer, '58) that, in human bein rather remarkable compatibilities may p vail between mother and child, in contr to tissue incompatibilities commonly served between father and child.

OPEN DISCUSSION

PAPAZIAN[1]: Just a small point conce ing the selective theory of antibody for tion. Is not three the critical number antibodies that cannot be produced b diploid cell, at least according to sc forms of the theory?

OWEN: If a single locus were involv of course the possibilities open to a dipl cell would be limited to two. I do not lieve that "triples" have ever been obser in cells from triply stimulated anim but probability considerations based on frequencies of single and double yield suggest that triples would not have b expected in the studies of this point date. In any case, the assumption o single locus seems gratuitous to me.

HIRSCHHORN[2]: So far as the vari comments on the placenta are concern two remarks might be of interest. On an experiment of Thomas, Douglas Carr, where they demonstrated in uterine veins of human mothers at vari degrees of pregnancy the existence of c trophoblast cells constantly entering circulation of the mother, which may v well be the mechanism of immunologi tolerance. They have also demonstra

[1] H. Papazian, University of Connecticut, Sto
[2] K. Hirschhorn, New York University G graduate Medical School.

at at least ABO red cell antigens do seem be present in these cells.

The inverse of this, the placenta as an tibody producer, may be also important. unsford has recently described a human imera, a woman who had two cell popu-tions. Her own was O and the twin hose cells she was carrying was an A. ie was married to a husband who was She had several pregancies, and in ch of the pregnancies the number of A lls in her blood diminished markedly and actically disappeared; she began to de-lop anti-A in her blood, which by theory fetal immunity would not be possible less the placenta was the organ that was oducing the antibody.

UPTON[3]: You referred to the work by artinez, Good, and associates on the ansfer of tolerance by parabiosis. It ould seem very important to determine hether cells or antigens of the histoin-mpatible strain in question were trans-rred from the tolerant animal to the condary recipient in the parabiotic rela-onship. Do you know whether cells or ntigens are present under these condi-ons?

OWEN: I do not know that this was one in studies by Good and his group. In e work that we did with parabiotic rats me years ago we were able to show an ex-hange leading to equilibrium of the two BC types in each partner within 4 days ter the operation. Dr. Pilgrim, who is ere, showed by fluorescene that there as a free vascular connection between e parabiotic mice he studied. I think we n assume that effective antigens did get ross.

PILGRIM[4]: I think there is ample evi-nce that, where parabionts have strong ntigenic differences, they are living to-ther in spite of these differences. In her words, there is a continuous immune action of some kind. The work on toler-nce in parabionts has been done with ani-als having comparatively weak antigenic fferences, or with animals rendered tol-ant by spleen injection into infant mice. this connection, I think that we have to nsider parabiosis as a rather stressful ocedure. It is quite possible that some of e tolerance observed might be a conse-quence of cortisone secretion as a response to stress.

OWEN: Yes, there are indications from other studies that animals placed under stress retain skin grafts significantly longer than do nonstressed animals. I also like the idea suggested by Rubin that humoral antibodies are being transferred that act in an enhancing system rather than true immunological tolerance.

HOECKER[5]: Dr. Owen has referred to the relationships frequently observed between psuedoalleles and complex systems of antigens. In reference to this problem, I would like to tell something about our findings in the *H*-2 system of antigens in the mouse that have led us to an interpretation more coherent with present ideas about the nature of gene organization.

The *H*-2 region in chromosome 9 of the mouse is concerned in the determination of a relatively high number of antigens (sometimes up to 10) inherited as one unit. A series of alleles at this locus has been described by G. D. Snell and his colleagues at Bar Harbor, by P. A. Gorer in London, and by us in Chile. When linkage studies are made, it can be shown that recombination between these antigens can occur in about 1% of the gametes, and crossing over at this region was consequently advocated by S. Allen, Gorer, and us.

The cross-over hypothesis was questioned in this same symposium by Dr. Owen in 1958 and also by Dr. Snell later on. In the meantime, more cases have accumulated, and it now seems to me that there are no good reasons to suspect it. The reciprocal products of crossing over have been obtained, and they hold the right relationships with neighboring genes; and in the following generations, the new "alleles" obtained through crossing over are stable.

This being so, we have to think of *H*-2 as being complex. But, to classify it as pseudoalleles, we have to resort to another criterion. Crossing over could indicate two closely linked but independent genes. This

[3] A. C. Upton, Oak Ridge National Laboratory.
[4] H. I. Pilgrim, University of Buffalo School of Medicine.
[5] G. Hoecker, Instituto de Biologia "Juan Noe", Santiago.

is not so, however, and the following observations indicate that, in spite of the relatively high frequency of crossing over observed, the *H*-2 region behaves as one physiological unit, i.e., one gene.

First, the distribution of *all* antigens determined by any one allele, say D, E, K, H, in different tissues is strictly correlated; viz., if an organ has a high content of the D it has also high contents of E, K, and H; if D is low, then all others will be low. Tissues, such as the brain or testis, that lack one antigen are lacking in all others, and so on.

Second, when we studied the stage of development at which these antigens would appear, we found to our surprise that they were not present at birth. Their first appearance would start about the third day. At this time *all* antigens in any one combination appear at the same time. During the following 3 days, *all* increased at the same rate and *all* reached the concentration found in adult tissues on about the sixth day. I would like to emphasize the strict correlation in time of appearance and rate of increase of *H*-2 antigens in ontogeny.

Third, this same situation holds for transplantation of fetal hemopoietic tissues into lethally irradiated hosts.

From all this, we believe the conclusion is that the *H*-2 region of chromosome 9 behaves as a "physiological unit" in the sense this word has been used by M. Demerec or S. Benzer. In other words, *H*-2 should be considered one locus. Incidentally, if this is so, the high percentage of crossing over found to occur between *H*-2 antigens would make it visible under the microscope, since a size of about 10,000 A would be obtained by applying the calculations made some time ago by H. J. Muller on the size of the gene.

It seems to me that the differences in antigenic specificity exhibited by the antigenic components of the *H*-2 complex reflect in some unknown way the structure of the gene itself; and perhaps a detailed knowledge of the chemistry of these antigens could be a strong weapon to attack the extremely difficult problems posed by the mechanisms of primary gene action.

AMOS[6]: Dr. Hoecker's point that the antigens tend to be inherited in a group is a very good one, but we did hear brie some discusssion of the one exception the antigen E on the C57BL red cell, wh there does seem to be a considerable pression in the red cell as compared other tissues. Then there is a very comp situation with some tumors. Some of less strain-specific tumors are genera poorly antigenic; but there does seem be somewhat of an upset ratio betwe certain of the components. It is theref possible to detect, say, a small amount the antigen F, whereas other antigens pected to be present may not be detectab I suspect this would be a very good sit tion to look for alterations in the antige This would also be a very good place attack chemically.

SILVERS[7]: I would just like to mak comment on the ability to transfer tol ance—and I hate to use this term— animals that are in parabiosis. Dr. B ingham and I have also tried to do th and in all our combinations tolerance rapidly abolished in the tolerant partn Of course we have used different str combinations from those who report s cess, and this must indeed be responsi for the different results obtained. I the fore think that we must bear in mind th what holds true for one genetic combi tion does not hold true for another. It a troubles me when we talk about this terms of the transfer of immunologi tolerance, when actually this has not be proved. I personally agree with Dr. Ow that probably one animal is tolerant wh the other may be enhanced.

AMOS: One aspect we have not talk about in this discussion is Dr. Owen's marks on rejection of certain paren tumors by an F_1. This is a very great fi of interest. There is one little note I wo interject here. We have been followi the reaction to a number of ascites tum injected into the peritoneal cavity of f eign strains of mice. The host's response these can be phenomenal. The respon seems to depend on the type of tissue jected, but also on the genotype of t host. A tumor like 60_3HED seems to p duce a prolific histiocyte response. The

[6] B. Amos, Roswell Park Memorial Institute

[7] W. K. Silvers, The Wistar Institute of Anato and Biology.

tiocytes seem to be quite active even in strain of origin, so that we would have be rather cautious in assessing the part yed by host cells.

OWEN: It seemed to me, from the im- nization evidence Snell cited, that this s not an immunological rejection but s probably related to some other physi- gical reaction. Dr. Amos's remark gives ossible basis for these otherwise vague siological interactions.

MARKERT[8]: My inquiry is directed to- rd the problem of whether the cell's notype is responsible for the kind of ibodies that it can make, or whether the l's genotype may be essentially irrele- nt. I have two considerations in mind. st, I understand that antibody-produc- cells can make antibodies against anti- ns that are synthesized by cells of the ne genotype. For example, is it not true t an individual can make antibodies inst its own lens antigens? Second, if genotype of the antibody-producing cell ermines its activities, then mutations such cells would place the organism in a y precarious position, because such tant cells would make antibodies inst the individual's own proteins. ese autoantibodies would lead to all ts of degenerative diseases as the mu- t antibody producers multiplied into ge clones and continued to manufacture ibodies against those proteins that, be- se of their mutant genes, they them- es could no longer synthesize and con- uently regarded as foreign. On the er hand, perhaps the truly significant ibute of an antibody-producing cell is embryonic history during which it rned" to recognize its own proteins. m this point of view, the problem is one cell differentiation and it is only coin- ence that antibody-producing cells nor- lly develop in individuals having the ne genetic makeup.

OWEN: I suppose that differentiation ains a riddle, which we may not solve n during this conference. The particu- kinds of differentiation that may occur he antibody-producing cell lineage, and possible role of somatic mutation, er directed or spontaneous, in this spe- kind of differentiation have been dis- sed elsewhere but remain hypothetical. I might mention, though, that there are instances of autosensitization and diseases of hypersensitivity, and some of these may be rather frequent and important. It is conceivable that they may relate to the mechanism you seem to imply should not exist.

WAELSCH[9]: In connection with this last remark, the existence of the BSVS strain in mice, which apparently shows susceptibility to experimentally produced encephalomyelitis as a result of the effect of two dominant genetic factors, seems to support the last point Dr. Owen made, namely, that perhaps this is not all a question of differentiation but that there is some genetic basis also for the autoimmune response.

AMOS: Dr. Owen actually made something of this point in his talk, where he was talking about differences in immunizability, in the ability to respond. There has been sporadic work in the past on this, and it seems to be a field that we really must get into. It is considered that inbred lines would be more or less uniform, whereas in fact this is not so. There has often been some selection of some particular character. Often there is heterozygosity for histocompatibility antigens or certain color genes. These things, which apparently have no selective advantages, are probably extremely heterozygous. I think we might define this much better by selective breeding procedures.

STERN[10]: I would like to underline Dr. Owen's remarks on the male-female histoincompatibility. We must be careful in ascribing specific histoincompatibility loci to the Y chromosome. At present we have proof of a male sex-determining Y-linked locus or complex of loci in mammals but of no other Y-linked loci. The assignment of histoincompatibility loci to the Y chromosome may be no more justified than the assignment of loci for moustaches or other male characters that only secondarily depend on the initial Y-dependent male determination during embryongenesis. There is also a possibility that the Y chromosome is not even indirectly involved in histoincompatibility, since this property may de-

[8] C. L. Markert, Johns Hopkins University.
[9] S. G. Waelsch, Albert Einstein College of Medicine.
[10] C. Stern, University of California, Berkeley.

pend on the quantitative 2X–1X difference between the sexes. This question may be attacked by transplantation tests involving XO mice that are females but carry only a single X chromosome.

RUNNER[11]: I am willing to rise to what I think was bait thrown out by Dr. Amos. We frequently hear allusions to residual heterozygosity in inbred mice. We all realize that the process of accumulation and of attrition of mutants during prolonged and continuous inbreeding assures some theoretically balanced but unknown level of heterozygosity. Evidence supporting the existence of heterozygosity is limited by the refinement with which we can assess the theoretical segregants. It is well known that, in inbred mice, the state of balance between mutation and attrition is accompanied with a surprising amount of phenotypic variation. Since analysis of variability is confined to detectable traits and since there is reason to suspect that phenotypic deviants are sensitive to minor genetic variations, Drs. Grüneberg, Searle, and Green —to mention just a few—have systematically classified and analyzed such deviants and shown that phenotypic variation *per se* does not prove the existence of genetic segregation.

It can be summarized for the record that, in spite of numerous attempts to demonstrate heterozygosity in inbred mice, a genetic basis for phenotypic variations within a restricted portion of a pedigree has utterly failed. It would seem therefore that the burden of proof for the significance of appreciable amounts of residual heterozygosity in inbred mice rests with those who would postulate its existence.

GRÜNEBERG[12]: We have recently investigated three inbred strains of mice for the presence of residual heterozygosity, using a whole battery of minor skeletal characters. The net result was that there was no evidence whatsoever for any residual heterozygosity in the strains CBA, A, and C57BL.

AMOS: I think one of the puzzling things is the wide diversity that has occurred in the C3H strain. This is tied up with the problem of heterozygosity in the inbred lines. Michie and McLaren have underlined the anatomical differences. We have shown antigenic changes in a number of sublines. On the other hand, have recently exchanged skin gr and tested hemagglutinins on DB and C57BL mice that have been separa for some 10 years. Skin grafts are taking after 9 weeks between the DBA/2 and the two C57BL sublines no antigenic differences were found.

E. S. RUSSELL[13]: Subline differer demonstrate that you do not have 10 and cannot count on ever having 10 homozygosity. By brother-sister inbr ing you get as close to 100% homozygo as you can. And then when you set sublines, if there is any residual het zygosity, it may become established. A we all know mutation may occur.

AMOS: On this question, I do not th we are going to prove heterozygosity un we deliberately look for it. Color and o obvious factors have usually been se tively eliminated. Heterozygosity in m minor factors is probably advantageo

FALCONER[14]: I should like to point one very simple fact that I think sig cant, but that seems to be ignored by th who say that there is much residual het zygosity in highly inbred lines. You can have a locus kept segregating permane in an inbred line unless both homozyg die or are sterile, because if one or other homozygote lives and breeds, e occasionally, you will sooner or later r two like homozygotes and so eliminate segregation—and perhaps the line This means that, for one locus to be permanently segregating, you must ularly lose half the offspring through t dying or being sterile. And if you h two unlinked loci permanently se gating, you must lose three-quarters of offspring. Now, I do not believe that could maintain a mouse strain in wh three-quarters of the offspring died or v sterile without noticing it, and co quently I do not believe that more t one locus, at the most, can show resi heterozygosity in our inbred mouse stra

[11] M. N. Runner, National Science Founda

[12] H. Grüneberg, University College, Lond

[13] E. S. Russell, Roscoe B. Jackson Mem Laboratory, Bar Harbor.

[14] D. S. Falconer, Institute of Animal Gen Edinburgh.

AMOS: Heterozygosity can have a selec-
ve advantage without the differences be-
g so extreme as lethality. A slight selec-
ve pressure has been suggested in quite
number of human blood group genes—
r example, in populations that do have
rather unexpectedly high incidence of a
rtain character. In inbred populations,
e tendency must be to eliminate many
these differences, but I suspect our ana-
tical techniques are inadequate to detect
y but easily demonstrated characters
d we know little about the selective ad-
ntages of so few of these.

FALCONER: A less-extreme selective ad-
ntage of heterozygotes can do no more
an slow down the rate of inbreeding.
will then take longer to attain a given
el of homozygosity, but the segregation
ll not be permanently maintained.

PILGRIM: Has anyone tested the color
netics of some of our albino strains?

DICKIE[15]: Color tests are continuously
rried out on albino stocks maintained in
r laboratory to insure against mutation
contamination.

PILGRIM: And you find that there are
incidents of heterozygosity, say, for
gouti genes in albino stock?

DICKIE: That is right.

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The Inheritance of Hemoglobin Types and Other Biochemical Traits in Mammals[1]

SALOME GLUECKSOHN-WAELSCH
Department of Anatomy, Albert Einstein College of Medicine, New York City

Interest in problems of mammalian genetics brings together investigators coming from a great number of different biological disciplines. There are those whose concern with mammalian genetics stems from a desire to study the mode of transmission of hereditary traits in mammals as compared with other organisms. Others are attracted by the particularly intriguing aspects of problems of population genetics of mammals. The cytogeneticist finds much interesting material in recent studies of mammalian chromosomes which are only beginning to open up new avenues of approach to problems of sex determination and abnormal development. For the embryologist, the use of gene-mediated deviations from normal development has proved most fruitful in the analysis of problems of mammalian morphogenesis and of causal mechanisms of mammalian development. There exist almost limitless possibilities of approach in mammalian material for anyone interested in the physiology of the gene, i.e., in the fundamental question of how a genetic change produces a change in form, function, or biochemistry of a trait. In this connection the field of transplantation genetics and mammalian immunogenetics reviewed by R. D. Owen (this symposium) is an example of perhaps one of the most active and productive areas in studies of mammalian genetics today. In all these many advances we seem to notice a hesitant return to the mammalian organism dreaded as an object for genetic research not so long ago because of its complexity.

It seems to me that, among the most significant studies of mammalian genetic research today, are those of the relation between genes and their products, which have placed the hemoglobins of man in the center of attention of many workers in the field of physiological genetics. It might be worth while to remember at this point that, in considerations of problems of gene action, a series of biochemical substances (e.g., pigments, enzymes, antigens, to mention just a few) have occupied central positions in the history of physiological genetics, and in every case one of the main questions revolved around the nature of the relation between the gene and these substances. How immediate was it and to what extent could the particular gene-controlled material be assumed to be a direct product of the biochemical entity carrying the genetic information? I shall not review here the physiological genetics of antigens and the impressive work of Irwin and others, but shall refer for this purpose to discussions by Goldschmidt ('55) and by Haldane ('54). I also am not planning to elaborate on the problem of gene-enzyme relationships and their interpretation from the point of view of gene action. I should like to put the main emphasis in this talk on a discussion of mammalian hemoglobins, the genetic and biochemical analysis of which has contributed much material for thought on gene action.

HUMAN HEMOGLOBINS

Eleven years ago Pauling and his collaborators ('49) published the first report of the hemoglobin abnormality in sickle-cell anemia, a hereditary condition in man. In the years following this report, much progress has been made in the analysis of the mode of inheritance as well as the biochemical basis of the various hemo-

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globin abnormalities in man. The subject matter has been covered in so many reviews that I shall not elaborate on the problems of human hemoglobins but only summarize the results most relevant to our discussion here:

1. More than a dozen different genes determining alterations of the globin portion of the hemoglobin molecule are known. These hemoglobins may occur together in different combinations; Harris ('59) reports 21 such known hemoglobin combinations, but even more exist today.

2. Differences in molecular structure with respect to the structure of the major component of normal adult hemoglobin have been analyzed in detail in five of the known human hemoglobins: S, C, E, G, and I. In each case they have been shown to consist of a replacement of one of the ~ 300 amino acids arranged in two polypeptide chains, α and β, in each half molecule of hemoglobin.

It was the interesting codominance behavior of the human hemoglobin genes that aroused our interest some years ago, since it seemed to indicate the existence of a fairly direct relation between genes and hemoglobins. In individuals heterozygous for A and S or C, both hemoglobins are present; none of these hemoglobin genes is dominant, nor does there exist a hybrid hemoglobin, a product of interaction of the parental genes. This situation seemed reminiscent of that found in many cases of genetic control of antigens where, for example, in the majority of blood group genes interaction of alleles fails to take place and both antigens are produced in heterozygotes.

MOUSE HEMOGLOBINS

Since we thought that an attack on problems of gene action with the help of such material as in the hemoglobins would be worth while and perhaps accomplished more easily in an experimental animal than in man, we proceeded to find out if different hemoglobins existed in the mouse and how they were controlled genetically. This work was done in collaboration with Dr. Helen Ranney of the College of Physicians and Surgeons of Columbia University, in whose laboratory all chemical experiments and observations reported he were carried out.

The original studies of mouse hem globins were done with filter paper elect phoresis, as used for human hemoglobi This method separates different hem globins from each other as a result of t different mobilities of such hemoglobi in the electrical field and has been ve valuable because of its simplicity. Wi the help of filter paper electrophoresis number of mutant and inbred mou strains were screened; two different hem globin types were found to be distribut throughout these strains (Ranney a Gluecksohn-Waelsch, '55). Hemoglob of some strains gives, upon filter pap electrophoresis, a spot that we call single whereas the hemoglobin of oth strains yielded a more diffuse spot consi ing of two or more components—a fast o with a mobility similar to that of the sing spot and a slower component; we called th single, type I, and the diffuse hemoglobi type II. Among the 11 strains studied, v found three giving the type I pattern onl five giving the type II pattern only, a three strains that contained some ind viduals with type I and some with type patterns (Gluecksohn-Waelsch *et al.*, '57

GENETICS OF MOUSE HEMOGLOBINS

Breeding tests

In order to test the genetic basis of thes hemoglobin differences, we made a nu ber of test crosses and subjected the hem globins of the offspring to filter pap electrophoresis. F_1 and F_2 as well as bac cross generations were tested (table 1).

All offspring from crosses of type II b type I showed the type II hemoglobi pattern. Offspring from type II by typ II gave type II hemoglobin pattern onl and offspring from type I by type I gav type I hemoglobin pattern only. The di fuse spot of the F_1 hybrids was indistin guishable by paper electrophoresis fro the diffuse spot of the parent.

Testcrosses of F_1 hybrids to the origina type I parent showed segregation of th two hemoglobin types in the backcros generation.

Intercrosses of F_1 hybrids from type I by type I showed segregation of type I

TABLE 1

Results of breeding experiments of mice with different hemoglobin types

Crosses (Types I and II)	No. of strains used	No. of offspring	Generation	Type II	Type I
II × I	7	101	F_1	101	0
II × II	2	23	F_1	23	0
I × I	3	16	F_1	0	16
F_1 II × I	6	102	BC	49	53
F_1 (II × I) by F_1 (II × I)	7	146	F_2	104	42
F_1 (II × II) by F_1 (II × II)	2	26	F_2	26	0
F_1 I × I by F_1 (I × I)	3	38	F_2	0	38
F_2 (II × I)					
(1)	7	37	BC	37	0
(2)	7	78	BC	45	33
F_2 (I × I)	7	68	BC	0	68

ıd type I in a 3:1 ratio in the F_2 genera-ɔn, but F_1 hybrids from either type II by pe II or type I by type I crosses failed to gregate. Finally, 19 offspring of the F_2 ıeration with a type II phenotype ob-ined from crosses of F_1 (II × I) hybrids ere tested in backcrosses to the type I ırent; seven of them failed to segregate ıereas 12 segregated for types I and II. one of the F_2 with the type I hemoglobin ıenotype segregated.

All these data are compatible with the sumption that a single gene difference termines the differences observed in ouse hemoglobins and that the genes de-rmining the types I and II are alleles. ıe phenotype of the hemoglobin patterns demonstrated in the filter paper electro-ıerogram was identical for the type II rent and the type II F_1 hybrid and made appear as though in the mouse, in con-ast to man, one hemoglobin allele showed minance over the other. The method paper electrophoresis did not, however, clude the possibility that in the F_1 the pe I pattern might still be present but asked by the type II pattern. It was erefore necessary to use other methods r the possible separation of the different moglobin components.

Starch electrophoresis of mouse hemoglobins

Vertical starch gel electrophoresis car-d out according to Smithies ('59) gave e results shown in figure 1A; a single moglobin band appeared in the case of moglobin type I from Kink. Hemoglobin type II from DBA included a band in the same position and in addition two slower and one faster components. The F_1 hybrid from type II by type I contained the main band visible in both parent types as well as the faster and the two slower bands of type II. Both the faster and the two slower bands were fainter in the F_1 than in the type II parent. The method of starch electrophoresis permits quantitative estimation of the relative amounts of hemoglobin components to a certain extent, and thus it was possible to show that the slow components in the type II hemoglobin measured about 20% whereas they amounted to only 4% of the total hemoglobin content in F_1 hybrids (Ranney *et al.*, '60).

Interestingly enough, the slow component increased in quantity upon storage of hemoglobin both in the type II (DBA) hemoglobin and that from F_1 hybrids (fig. 1E). Such slow components never showed up in hemoglobin specimens of type I from Kink, even after several weeks of aging. Further studies of the phenomenon of increase of the slow component in type II and F_1 hemoglobin indicated that this was the result of aggregation of part of the hemoglobin into larger and therefore more slowly migrating molecules. In contrast to type II hemoglobin, that from F_1 mice showed a strong main band even after aging, indicating the presence of type I hemoglobin in the F_1 hybrid. Results of ultracentrifugal analyses of hemoglobins agreed with the starch gel electrophoresis results in showing in type II and F_1 hybrids an increase in amount of rapidly sedimenting component

probably identical with the slow component of starch electrophoresis (fig. 1).

These results may be interpreted in the following way:

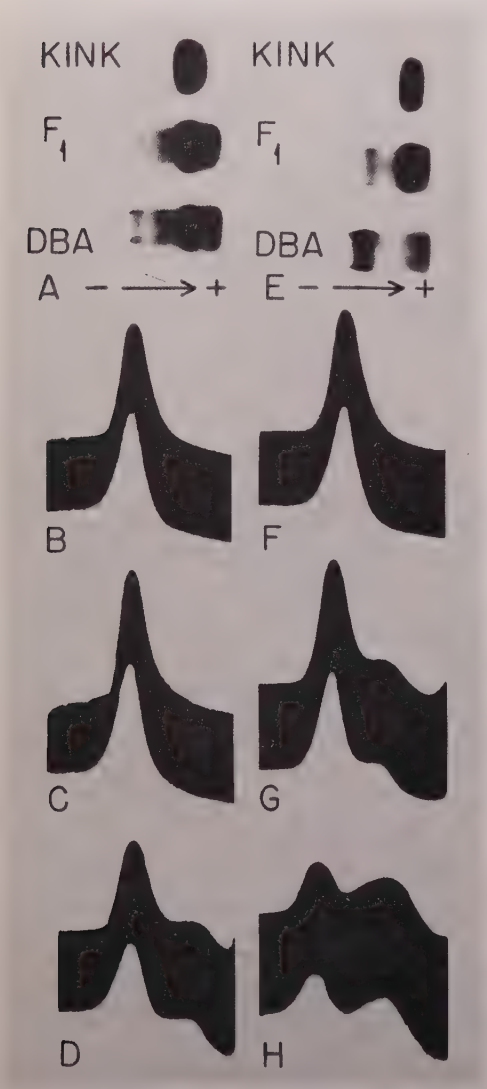

Fig. 1 Electrophoretic and ultracentrifugal analyses of carbonmonoxyhemoglobin of inbred strains of mice (Ranney *et al.*, '60).

A, Initial vertical starch-gel electrophoresis of Kink, F_1, and DBA hemoglobin; E, electrophoretic analysis of the same solutions after 4 weeks of storage at 4°C.; B, C, and D, initial sedimentation diagrams of Kink (Type I), F_1, and DBA (Type II) hemoglobins, respectively; F, G, and H, sedimentation diagrams of same solutions after 4 weeks of storage.

Hemoglobin type I from Kink sho homogeneity under various conditions in cating the existence of one type of mo cule.

Hemoglobin type II from DBA contai several components, one or more of whi possess the ability to aggregate.

The F_1 hybrid seems to contain a mi ture of these two hemoglobin types.

It should be emphasized at this po that the results reported here were obtain with type I hemoglobin derived from t inbred mutant strain Kink, and with ty II hemoglobin from the inbred strain DB 1. There are indications that not all ty I hemoglobins as identified by paper elect phoresis show identical starch elect phoresis patterns nor that all type II hem globins are alike. It is conceivable th other type I hemoglobins may conta more than one molecular species, and th other type II hemoglobins may be co posed of fewer or more different molecul than the particular type II studied by

Strain differences

After our original demonstration of t genetically determined hemoglobin typ several other studies reported inherit electrophoretic hemoglobin patterns mice. Russell and Gerald ('58) found of 20 inbred mouse strains to have the ty II hemoglobin and 6 the type I, a distrib tion agreeing well with that reported or inally in our strains; there seem to be a proximately twice as many strains wi the type II hemoglobin pattern as tho with type I. No selective advantage seem to be associated with either hemoglob type. Welling and van Bekkum ('58) r ported electrophoretic studies of mou hemoglobin in two inbred strains, one which has only one component, the oth two. It is not possible to tell from the paper the nature of the F_1 hemoglobin, though it may contain a mixture of the t parent hemoglobins. Rosa *et al.* ('58) r ported the existence of at least 4 hem globin patterns in 6 strains of mice studie Their brief report does not allow evalu tion of their data in terms of uniformity patterns within strains, reproducibility patterns, or possible changes with aging discussed above.

None of these authors reports an asso-
ation of hemoglobin type and anemia
s is the case in man.

ANALYSIS OF GENETIC DATA OF MOUSE HEMOGLOBINS

With these data at hand, we may try to
nalyze the genetic basis of mouse hemo-
lobins and compare it with that of man.
pair of alleles, Hb^1 and Hb^2, seems to
etermine the presence of either type I or
ype II hemoglobin. Filter paper electro-
horesis indicated dominance of Hb^2 over
b^1 since only type II hemoglobin could
e demonstrated in the F_1 hybrid. Starch
lectrophoresis, however, revealed the pres-
nce of a mixture of hemoglobins in the
hybrid, indicating that Hb^1 and Hb^2 had
ne same codominance relationship as the
enes for hemoglobins S and C in man. In
ddition, starch gel electrophoresis demon-
rated the existence of considerable molec-
lar heterogeneity in hemoglobin type II;
lso the storage phenomenon with subse-
uent aggregation could best be explained
y assuming the existence of more than
ne type of hemoglobin molecule in type II.

We wonder how this kind of genetic con-
ol of hemoglobin formation in the mouse
ts in with the scheme proposed by Ingram
59; Ingram and Stretton, '59) for hemo-
obin control in man.

In man, at least two genetic loci deter-
ining hemoglobin seem to exist. The
enes for hemoglobins S and C are thought
genetic evidence to be alleles at one of
ese loci (Ranney, '54), all three alleles,
e., A, S, and C, showing the phenomenon
codominance, so that individuals hetero-
gous for any two of these hemoglobin
enes express the presence of both cor-
sponding hemoglobins phenotypically.
he statistical evidence indicating allelism
S and C received strong support from
ie finding (Hunt and Ingram, '59) that
and C alter the identical amino acid in
ne of the peptides of the β chain, which
suggestive of allelism of S and C, if we
ssume a direct relation between linear
ructure of the gene and linear arrange-
ent of amino acid residues of the hemo-
obin molecule.

Whereas the mutations S and C are lo-
ted at the same place of the genetic unit controlling synthesis of the entire peptide, G has been reported to be genetically independent of this locus. An interesting and at the same time puzzling fact in this connection lies in the demonstration that in hemoglobin G the amino acid immediately adjacent to that changed by S and C is altered (Hill and Schwartz, '59).

The genetic evidence for allelism or close linkage of E and S is not very good; the chemical difference between E and normal hemoglobin consists of a change of one amino acid in the β peptide chain of the E hemoglobin (Ingram, '59). The fact that in E also the β chain is affected, suggests to Ingram linkage of E and S. The molecular change in hemoglobin I has been reported as a substitution in the α chain by Murayama and Ingram ('59).

The discovery of a distinct molecular change, i.e., the substitution of a single amino acid in the different hemoglobins, resulting from a single genetic difference has led to much speculation about the nature of the genetic change and of gene action.

Ingram ('59) has formulated the genetic basis of hemoglobin synthesis as follows: There exist two sets of genes—(1) α genes controlling the synthesis and the amino acid sequence of the α chains, with hemoglobin I representing an example of α chain mutation, and (2) β genes controlling synthesis and amino acid sequence of the β peptide chain of the hemoglobin molecules, with hemoglobins S, C, E, and G representing β-chain mutations.

Consequently, the normal genotype would be: α^A/α^A β^A/β^A ; that of S, α^A/α^A β^S/β^A or α^A/α^A β^S/β^S; and that of I, an α-chain mutation, α^I/α^A β^A/β^A or α^I/α^I β^A/β^A.

A number of questions arise in connection with such a scheme. We wonder, for example, why the hemoglobin G gene should be genetically independent of the gene for S hemoglobin, although the changed amino acid is immediately adjacent to that affected in S. In view of this close proximity, recombination between these loci, would be expected to be a very rare event. Another question concerns the genetic basis of human hemoglobin heterogeneity as expressed, for example, in the

presence of the A_2 component in normal hemoglobin.

If we now look once more at the results of genetic studies of mouse hemoglobins we notice on the one hand, in the mouse as in man, that the hemoglobin genes express themselves independently of their allelic partners and each forms its own hemoglobin. Thus the idea of a close relation between gene and gene product in the case of hemoglobins receives support from these studies. On the other hand, several components with different electrophoretic mobilities exist in the type II mouse hemoglobin, a fact that does not fit easily into the scheme proposed by Ingram for the genetic control of human hemoglobins. It is of course difficult to speculate profitably on gene action in the case of mouse hemoglobins in the absence of any further knowledge of their chemistry. Thus, for example, we do not know if mouse hemoglobin actually contains two different polypeptide chains. The phenomenon of the existence in type II mouse hemoglobin of several molecular species is puzzling and indicates a heterogeneity most likely genuine and not the result of physicochemical artifacts.

A number of different hypotheses could be advanced to account for the observations; among them the following scheme is proposed in full awareness of its highly speculative nature. Justification for it may be found in the fact that it lends itself to experimental verification.

Suppose there existed in the mouse originally two gene loci for hemoglobin in analogy with man. One of these loci (e.g., the one controlling the β chain) might be assumed to have undergone duplication several times resulting in a series of two or more neighboring genes all with the function of controlling hemoglobin synthesis. A possible selective advantage of such an arrangement resulting in perpetuation of this genotype might be seen in the fact that the production of normal hemoglobin would remain assured even if a mutation in one, two or more of the series of duplicated genes interfered completely with hemoglobin synthesis at the mutated locus as long as one of the loci remained undisturbed. Some mutations these duplicated loci may lead, not to su pression, but to the production of hemog bins, modified and only slightly differe from the normal molecule and fully fun tional.

Thus several molecular species of hem globin may be produced by the differe loci, each with slightly different phys logical properties, in respect, for examp to oxygen-binding capacity. Such a con tion might confer an adaptive advanta on the carrier since the organism wou have available a variety of hemoglobins meet various specific requirements.

The role of chromosomal duplicatio in providing a "reservoir" of extra gen from which new ones may arise has be stressed by E. B. Lewis ('51) and go back to Bridges' analysis ('36) of the Bl eye mutation. The originally identic genes resulting from duplications becom different from each other as the result mutation and may form a complex locu The hemoglobin locus in the mouse m thus resemble the Rh locus in man or t blood group locus in cattle.

This highly speculative scheme is ope to experimental verification by furth physicochemical and physiological studi of mouse hemoglobins, which we hope be able to continue in collaboration wi our colleagues.

The genetic locus controlling synthes of one of the polypeptide chains of mou hemoglobins may be visualized perhaps illustrated in figure 2.

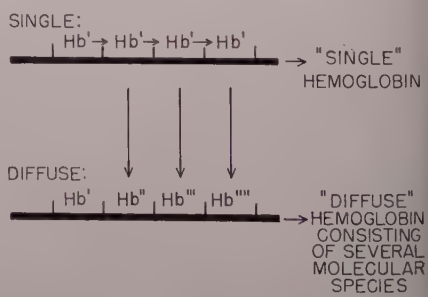

Fig. 2 Diagram of gene arrangement in typ I "single" and II "diffuse" hemoglobin.

The different hemoglobin molecules pre ent in the diffuse type might be made u

the following combinations of peptide ains, with corresponding genotypes:

Hemoglobins	Genotypes
$\alpha_2\beta'_2$	$\alpha/\alpha\ \beta'/\beta'$
$\alpha_2\beta''_2$	$\alpha/\alpha\ \beta''/\beta''$
$\alpha_2\beta'''_2$	$\alpha/\alpha\ \beta'''/\beta'''$
$\alpha_2\beta''''_2$	$\alpha/\alpha\ \beta''''/\beta''''$
etc.	etc.

One of the tests of this hypothesis would nsist in demonstrating by chemical eans different β chains in the type II moglobin which contains several molec- ar species.

Another verification of this hypothesis uld come from genetic data of crosses volving different type II and different pe I hemoglobins in which segregation ight occur.

Heterogeneity of hemoglobin exceeding at expected on the basis of the assump- n of a 1:1 relation of genes and hemo- bins exists also in man. First of all, there the well-known presence of a minor mponent, A_2, in normal hemoglobin, as scribed by Kunkel and Wallenius ('55). ven further heterogeneity in normal he- oglobin is reported by Harris ('59). The monstration of a single amino acid ange in the A_2 component of normal man hemoglobin (Stretton and Ingram,) seems particularly interesting in view the failure to separate A_2 from the main mponent of human hemoglobin genet- lly. This may be suggestive of the pres- ce of similarly duplicated adjacent he- oglobin genes in man, as postulated in e mouse.

That a human individual heterozygous r α and β genes may have more than the ree expected hemoglobins, and as many 4 molecular species of adult hemo- bin in his red cells, has been demon- ated by Itano and Robinson ('59, '60). ese authors showed the *in vitro* forma- n of normal and doubly abnormal he- oglobins by molecular recombination of moglobin I (an α-chain mutant) with her S or C (β-chain mutants). They so demonstrated the presence of 4 molec- ar species in the hemoglobin of indi- duals heterozygous for Ho-2 (α-chain utant) and S (β-chain mutant).

HEMOGLOBIN DIFFERENCES IN SHEEP

Genetically determined hemoglobin differences have been described also in sheep (Evans *et al.*, '57). Two allelic genes, A and B, seem to exist, each responsible for the formation of one kind of hemoglobin and showing codominance. Gene frequencies of A and B vary among breeds of sheep, particularly between lowland and mountain breeds, indicating possible adaptive significance of the hemoglobin types.

Physical and chemical differences between these sheep hemoglobins have been investigated and are rather numerous (Huisman *et al.*, '59); amino acid composition has been described as varying quantitatively. It should be stressed that these studies determine quantitative and qualitative aspects of total amino acid composition but do not carry out analysis of amino acids as those done by Ingram in human sequential hemoglobins. The two types of sheep hemoglobin show more pronounced changes in oxygen affinity.

HEMOGLOBIN DIFFERENCES IN CATTLE

The mode of inheritance of hemoglobin types A and B in cattle was studied by Bangham ('57), who showed that the different hemoglobins were controlled by a pair of alleles showing codominance. Among the breeds studied, the frequency of hemoglobin A was considerably higher than that of B. As in other mammals, and in contrast to man, no association of hemoglobin types with disease was found.

LACTOGLOBULIN DIFFERENCES IN CATTLE

Further examples of hereditary structural variations in proteins of mammals other than man are present in lactoglobulin formation in cattle, where the genetic basis resembles that of hemoglobins (Aschaffenburg and Drewry, '55). Two different β-lactoglobulins, A and B, have been reported, determined by a pair of alleles showing codominance. The two lactoglobulins differ in the number of titratable carboxyl groups (Tanford and Nozaki, '59).

β-GLOBULINS

Ashton and McDougall ('58) report polymorphism involving the β-globulins of cattle, sheep, and goats. The genetic basis of β-globulin control in cattle has been found to lie in a three-allele system with codominance, so that 6 phenotypes may be distinguished. Goats were shown to carry two alleles with codominance and therefore yielding three phenotypes. The most complex genetic mechanism, involving a series of 5 alleles, was reported for β-globulin polymorphism in sheep.

OTHER BIOCHEMICAL VARIATIONS

Whereas qualitative changes in protein molecules are the result of mutational changes in the characters discussed so far, there do exist many hereditary biochemical changes in which the mutations express themselves in variations not directly traceable to qualitative changes of proteins.

Having devoted, however, by far the largest portion of this paper to hemoglobins, I have little time left to discuss other biochemical traits in mammals. Perhaps all that can be done at this point is to select a few examples particularly interesting for this discussion for various reasons.

Potassium level in sheep erythrocytes

Evans *et al.* ('56) reported genetic variation of potassium concentration in the red blood cells of sheep. High potassium is associated with low sodium concentration, and low potassium with high sodium. These phenotypes are determined by a single gene pair with high potassium being recessive. The genes for sheep hemoglobin types and potassium concentration assort independently, but some physiological association seems to exist between hemoglobin A type and high potassium concentration.

It should be emphasized that the consideration of biochemical variations in mammals presented here makes no claim for completeness. As a last point, I would like to stress one unexpected and striking observation that emerges from a study of recent reports of some genetically determined enzyme abnormalities in mammals. Whereas the student of hereditary morphological traits is fully aware of the spatial and temporal distance by which the t under study is removed from gene act studies of enzyme variations are freque affected by the prejudice that they c necessarily with events in close proxim to the gene. It might be worth while cite several studies here in which gene ally caused enzyme abnormalities w shown to be caused not directly by altered gene but to be a secondary resul other gene effects.

β-Glucuronidase in mice

Studies of genetic variation of the li enzyme, β-glucuronidase, in mice h shown that a recessive gene determine deficiency of this enzyme in some mo strains (Paigen, '59). It should be no that, according to Paigen this deficie is correlated with a decrease in amoun enzyme rather than with an alteration the enzyme. No differences in physica kinetic properties of enzyme preparati from normal and mutant animals could found. There also was no evidence for presence of activators or inhibitors. T author assumes that two different ge are involved possibly linked to each ot —one responsible for the production enzyme, the other controlling its amou

Congenital jaundice in rats

Similar variations in quantity rat than quality of a genetically affected zyme have been demonstrated by Axel *et al.* ('57) in rats with congenital ja dice. As a result of this recessive mu tion, a defect is found in the conjugat of bilirubin with glucuronic acid, a nec sary prerequisite for its normal excreti This defect results from a decrease but an absence of activity of the enzyme g curonyl transferase, as shown by *in vi* synthesis of glucuronide with liver mic somes from normal and mutant anim Such synthesis was depressed in exp ments with mutant microsomes but not sent and was not caused by the prese of inhibiting factors in the mutant li microsomes.

Glucose 6-phosphate dehydrogenase

In connection with reports of genetica controlled variations of glucose 6-ph

nate dehydrogenase activity in human rythrocytes, we undertook some time ago study of this enzyme in different mouse rains. The results of these studies are as t inconclusive, but several points of in- rest emerge. First of all, variations of ctivity of this enzyme in the mouse strains udied have been demonstrated clearly. owever, they are of quantitative nature ther than indicating qualitative varia- on of the enzyme. Recent results of stud- s of the same enzyme in human red blood ells (Rimon *et al.*, '60) show that the enetic differences in man cannot be ex- ained by qualitative changes of the en- me itself but rather result from the con- ol of its activity by some other factor siding in red cell stroma. It is conceiv- ble that a similar situation exists in the ouse too. One of the interesting features this work is that it provides another emonstration of a distant relation be- veen gene and enzyme.

CONCLUSION

I have attempted in this paper to dis- ss some possible interpretations of re- lts obtained in studies of the genetic ntrol of hemoglobins and of some en- mes in mammals other than man. Stud- s of mammalian hemoglobins seem to dicate a close relation between genes and emoglobins, making them valuable mate- al for analysis of relationship between enes and proteins. As I said in the begin- ng, the hemoglobins occupy today a cen- al position in the interest of workers con- rned with the physiology of gene action. ut the relation between genes and hemo- obins may actually be considerably more mplex than now conceived. In the halysis of human hemoglobins, there are ready appearing on the horizon the ouds of hemoglobin G and of molecular eterogeneity. Nevertheless, it seems to e that in the studies discussed the con- rted efforts of geneticists, biochemists, nd other scientists in the attack on prob- ms of genes and hemoglobins have set a ttern for future research in mammalian enetics, which promises to lead us fur- er toward our understanding of the hysiology of the gene.

OPEN DISCUSSION

GREEN[2]: Have you considered testing the duplication hypothesis by looking for crossing over within this region? This locus is in the first linkage group, is it not? Are there suitable markers on opposite sides of it so this would be reasonably easy to do?

POPP[3]: I have data that indicate that the hemoglobin locus is in the first linkage group. A couple of years ago Dr. W. St. Amand and I undertook a study to learn whether the hemoglobin locus is compound. The position of the locus had to be determined first. Linkage studies indicate that the position of the hemoglobin locus is about 4 crossover units from albinism and one crossover unit from shaker-one; the linear order is pink eye, albinism, shaker-one, and hemoglobin. Thus there are markers within a few per cent crossover on the one side, and frizzy is on the other side, but it is somewhat more distant —about 16% crossover. With the aid of these markers, a number of animals could reasonably be examined to determine if the locus is compound, as Dr. Waelsch has suggested. I would like to add that the various types of hemoglobins found in laboratory mice have also been found in a small sample from a population of wild mice we have examined.

HIRSCHHORN[4]: As one who limits himself to a different kind of mammal than most people in the audience here—namely, man—I wonder if I might make a few comments on what Dr. Waelsch has said. First of all, there exists in man a protein that seems to be genetically determined, as analyzed by Smithies and Harris. It is called a haptoglobin, which is an α-globulin that binds hemoglobin in the serum. Basically, three types of individuals have been found; one of them has so-called haptoglobin-1 (Hp^1), which on starch-gel electrophoresis shows one broad band, similar to the simple hemoglobin. Then there is a haptoglobin-2 (Hp^2), which shows about 6 or 7 (depending on how accurately you

[2] Margaret C. Green, Roscoe B. Jackson Memorial Laboratory.

[3] R. A. Popp, Oak Ridge National Laboratory.

[4] Kurt Hirschhorn, New York University Postgraduate Medical School.

measure it) different bands—very similar to the situation in the diffuse hemoglobin. Homozygous Hp^1 will have just this one band. Homozygous Hp^2 will have these. The heterozygote will show a thin band corresponding to haptoglobin-1 and a number of bands corresponding to Hp^2 but slightly faster in movement than these bands, on accurate measurement.

Incidentally, it struck me that this variation of expression of the multiple band component may be a possibility in some of your starch-gel diffusion patterns, too. Smithies was greatly puzzled by this, and the same type of theories have been postulated. He tried to find out what was going on. When he put into the starch gel three substances that break the three classical polymerization bonds—formate to break hydrogen bonds, urea to break ionic bonds, and thioglycollate to break disufide bonds—he again got a single band for homozygous Hp^1. However, homozygous Hp^2 had just a single, slow-moving broad band and the heterozygote showed two bands identical to these two. In view of that, the possibility then exists that the diffuse hemoglobin fractions may be polymers. This also might have some support in the fact that in man, when a hemoglobin is permitted to age, a fraction appears called A-3 hemoglobin, which is most likely a polymer A hemoglobin. It travels also slightly differently from A hemoglobin.

Another comment on the question of the differences between the various types of single or diffuse hemoglobins in mice—I think a good example again may be taken from man. There are several types of so-called D hemoglobins in man where one has been shown to have a defect on the α polypeptide chain, the other on the β chain. Yet electrophoretically, they look completely alike because there is no charge difference.

It might be of some interest to mention that fetal hemoglobin also has two half chains, each of which consists of two polypeptides. It contains the same α polypeptides, but to this is hooked on a different one that has been called γ, which has recently been of great interest because a totally different kind of human hemoglobin has been discovered—so-called human hemoglobin H. This consists, instead of two α and two β chains, of 4 identical chains. There is also a fetal hemoglobin that has 4 identical chains. One of them has 4 γ's and the other 4 β's, but I am not sure which is which.

MARKERT[5]: Was that in a fetus?

HIRSCHHORN: This was in an adult.

WAELSCH: To start with the last comment, actually hemoglobin H, according to my latest information—which I think is from the Federation Proceedings—does not have γ chains, but 4 β chains, i.e., complete absence of α chains. It is β_4.

I did not discuss fetal hemoglobin. I am aware that fetal hemoglobin has β chains but has α chains and in addition γ chains, but that will lead into a different area. We did not study fetal hemoglobin in mice.

As to the first comment on the polymer, we were aware of Smithies' work and have actually discussed this with Smithies. His methods, when tried on diffuse hemoglobins, did not give results analogous to those with the haptoglobins. Where there may be a chance that one or two of the bands actually represent polymers, there are still two bands left that have different heterogeneity. I was very glad that you brought up the point that there may be similarity in charge and still a difference in molecules.

E. S. RUSSELL[6]: Dr. Waelsch, have you found any evidence of fetal hemoglobin in the mouse?

WAELSCH: I personally have had no experience with fetal hemoglobin. I believe Dr. Ranney, who has studied fetal hemoglobin, has told me that as far as alkali denaturation goes, it behaves much like human fetal hemoglobin. She has been trying to find out whether the fetal hemoglobin from type I is different from fetal hemoglobin from type II. But as far as I know, she has no conclusive answer to this question.

E. S. RUSSELL: But she does have a difference between what she gets from fetuses and from adults?

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[5] C. L. Markert, Johns Hopkins University.

[6] E. S. Russell, Roscoe B. Jackson Memorial Laboratory.

WAELSCH: Yes, I believe so. It is hard to work with fetal hemoɔbin of the mouse because of the size of e fetuses; but there is definitely a difrence with respect to denaturation.

SILVERS[7]: Dr. Waelsch, you mentioned at, in some of the inbred strains tested, u found indeed both types. How inbred e these strains?

WAELSCH: They were not highly inbred. a matter of fact, they were just line ɔred strains, and there seemed to be a od correlation between the degree of ineeding and just one type of hemoglobin, d lack of inbreeding and presence of o types of hemoglobin.

STRONG[8]: Apparently there are several nds of anemia in mice. Has anyone done y work to distinguish this type?

WAELSCH: Dr. Russell has done some of is work. In *W* anemia, affected and noral sibs have the same hemoglobin type.

POPP: I would like to comment on a cent observation in reference to the exession of flexed anemia—not that the moglobin is necessarily different. The ect of the *W* genes is expressed in erythcytes produced in the fetal liver and also the adult bone marrow. With the flexed ne, erythrocytes produced in the fetal er are affected, but the bone marrow es rise to normal erythrocytes. Red ls derived from the fetal liver are slightly ger (8 μ) than those from bone marrow μ in diameter). The former cell is also ghtly hypochromic and contains siderin nules. Among a partially inbred group flexed mice that I obtained from Dr. zabeth Russell, there was one with a gle type of hemoglobin. When fetal er of flexed mice was injected into Xadiated adults, the fetal liver seeded out the bone marrow. Although the initial ls derived from the graft were about 8 μ diameter and slightly hypochromic, erin granules were lacking when these ls differentiated in the bone marrow enonment as opposed to their normal deopment in the fetal liver site. Thomas d associates have recently shown that formation of human fetal hemoglobin nilarly is partially dependent on the enonment of the fetal tissue.

MARKERT: In connection with the hypothesis that hemoglobin-synthesizing loci might be duplicated, I should like to mention that Bangham has shown that the horse produces two distinct hemoglobins that cannot be attributed to heterozygosity. This implies that there are two independent loci, each of which is synthesizing its own hemoglobin. When horses were mated to donkeys, to produce mules, it was found that the two horse hemoglobins were inherited together; the mule inherited both horse hemoglobins and probably also a distinct donkey hemoglobin.

Dr. Waelsch, why do you consider the G hemoglobin to be a disturbing exception to the one gene, one polypeptide relationship?

WAELSCH: If one follows Ingram's hypothesis, the finding that G and S are independent genetically is rather unexpected in view of their effects on two neighboring amino acids in the same polypeptide.

MARKERT: Can this be explained as a mutation of the locus?

WAELSCH: Perhaps, but they would still be close enough so that crossing over would be an extremely rare event. Only one family has been studied; but an individual heterozygous for G and S gave several normal children who had neither G nor S hemoglobin. The possibility exists (and this is what is being followed up now) that the G referred to in the pedigree is not the same G as the one that has been studied biochemically. It is a rather complicated situation. In short, the G investigated in family studies is not really the same G as that shown biochemically to have one amino acid change. This is being studied further. But if the G's are, indeed, the same, then this would be hard to explain.

MARKERT: Of course, the reliability of human breeding data is somewhat less than that for mice, especially when one is concerned with only a single family.

FRACCARO[9]: I want to mention briefly another aspect of biochemical research in mammals, namely the study of hapto-

[7] W. K. Silvers, The Wistar Institute of Anatomy and Biology.

[8] L. C. Strong, Roswell Park Memorial Institute.

[9] M. Fraccaro, University of Uppsala, Sweden.

globin types in primates. The protein patterns obtained by starch-gel electrophoresis are similar to human patterns, but recent studies (Arends and de Rodriguez, '60; Mäkelä *et al.*, '60) of small samples of Primates (mostly *Macacus rhesus* and *Macacus mulatta*) revealed the presence in all individuals of a pattern similar to the human Hp^1/Hp^1 type. This led people to wonder whether there is in Primates polymorphism for haptoglobin types. This question has important evolutionary implications. Dr. L. Beckman of Uppsala has now found two instances of Hp^2/Hp^1 types of *Macacus irus* and moreover obtained evidence that there is in Primates also polymorphism for transferring variants.

WAELSCH: I saw a report referring to some unpublished data of Dr. Dunn. I was wondering whether any more has come out on the B_2 allele found in some family studies and affecting the minor component of normal human hemoglobin about which Ceppelini spoke. I think it would be extremely interesting to find a mutation of A_2 to B_2 that is not affecting the main component of normal human hemoglobin. Have you any more data on that?

DUNN: If I might speak for Ceppelini and Tunkel, they are arguing it out next week.

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Phenogenetic Aspects of Some Hair and Pigment Mutants[1]

HERMAN B. CHASE AND STANLEY J. MANN
Biology Department, Brown University, Providence, Rhode Island

There is a prominent area of investigation in the field of mammalian genetics known as "phenogenetics." One portion of this area is particularly amenable to study, since it deals largely with developmental and physiological phenomena that are already relatively well known in mammals and other vertebrates. A related portion of phenogenetics that deals with the biochemical steps underlying these phenomena, and thus is closer to the genes, will in turn become more amenable to study as these phenomena and their defects become better known through observation of the variations attributable to mutant genes and teratogens.

In the hair and pigment mutants, most of the pertinent developmental and physiological processes can be observed in late fetal, postnatal, or even adult animals, which should facilitate the future associated biochemical studies aimed at closing the gap between the gene and the definitive phenotype. Most of the examples of mutants and teratogenic effects chosen for presentation here are taken from the mouse and represent only illustrative samples, not an exhaustive survey (table 1). A comprehensive scheme of developmental and physiological phenomena, relating directly to only that portion of phenogenetics more or less immediately preceding the final phenotype, will be suggested in connection with these examples.

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TABLE 1

Organizational scheme for selected examples of hair and pigment mutants and teratogenic effects

All examples from mouse except where otherwise indicated

Structure and mechanisms	Hair mutants	Teratogenic effects
Follicle initiation	Ragged, crinkled, Tabby	X-ray block
Follicle differentiation	Ragged, crinkled, Tabby, fuzzy	
Follicle orientation	Ragged, Rough (guinea pig)	Wound slope
Follicle curvature	Ragged, Rex?	Wound curvature
Bulb mitotic activity	Ragged, crinkled, Tabby, fuzzy	Colchicine reduction
Bulb structure		X-ray bulb disorganization and epilation
Keratinization of hair	Wuzzy (rabbit), Naked, Rex?	Biotin epilation, X-ray filum
Keratinization of club	hairless, furless, tufted	X-ray block
Migration of hair	new variant?	X-ray reduction
Regeneration cycle	hairless, furless, angora (guinea pig and rabbit)	X-ray block
Stem cells	piebald, silver, dom. White	X-ray graying, early explants
Mature melanocytes	Light, silver	X-ray hyperpigmentation and disorganization
Granules	br, Blk, pp dil, Yellow, Agouti	
Melanization of granules	br, Blk, pp dil, Yellow, Agouti, albino	Biotin graying, transplant to new area
Clumping of granules	*d/d* dilute	
Transfer of granules		X-ray graying?

HAIR

General description. Certain aspects of hair follicle development and morphology are known or postulated (Chase *et al.*, '51; Chase, '54, '58a, b) and various hair mutants and teratogenic effects on normal individuals lend evidence for or against the postulates. In fact, certain deviations can even be predicted on the basis of known steps and requirements in normal development. In the original initiation and differentiation of the follicle, there is a proliferation from the basal layer of the epidermis. Those proliferations, which appear earlier in the mouse, produce the larger and more widely spaced follicles (monotrichs and awls), whereas those initiated later develop into the smaller ones (auchenes and zigzags), which fill in the spaces between the others. The time span for initiation of pelage hairs is from 5 days before birth to at least 3 days after birth. During that period the follicle-producing potential of the cells of the basal layer changes considerably, the cells being competent to produce large monotrichs or small zigzags only at certain stages. It is also possible that the presence of earlier follicles influences the production and type of subsequent follicles.

After initiation of follicles, there normally is differentiation and elongation, the expanded and dimpled tip of the projection forming the bulb and enclosing the mesenchymal cells, which become the dermal papilla. The epithelial matrix cells of the bulb then produce the cells for the layers of the inner sheath and the hair. The outer of the concentric layers of the inner sheath, the Henle layer, keratinizes early and may serve as a funnel for arranging the other layers. The degree and type of keratinization of the hair are determined in the keratogenous zone above the bulb, but they may also depend on the number and distribution of the cells arriving from the bulb. Straightness or curvature of the shaft depends possibly on the types of keratin formed and possibly also on the curvature of the upper follicle where hardening is completed.

The movement or "rate of growth" of the hair, although usually considered a function of mitotic activity in the bulb, is probably a result of migration of the inner sheath outward along the external sheat The hair is carried along to the sebaceo gland and canal region by the interlock cuticles of the hair and inner sheath. this point the inner sheath disintegrates a column. The cells from the bulb th contribute to the hair shaft merely fill t space behind the outward-moving sh but do not push the shaft. The norm zigzag hair has two or three constrictio with a missing or reduced medulla at the constrictions, a single row being prese elsewhere. After emergence, the ha bend at these spots, thus giving t name to the hair type. The normal a chene has only one constriction. Rega less of contrictions, however, migrati proceeds at a steady rate even though t supply of cells is periodically reduced, in cating again that the rate of emergence the shaft is not a result of the cells fr the bulb exerting pressure.

A most important aspect of hair folli growth and development is the hair generation cycle. In catagen there a changes that transform a follicle from hair-producing phase (anagen) to the re ing phase (telogen) ready for the ne growth. Mitoses in the bulb cease, anchoring keratinized club is formed, t follicle shortens tremendously, and la the shaft ceases its outward migratio From the "germ" and upper exter sheath, a new elongation and different tion produces an active follicle that in tu produces a new hair, the next hair gen ation.

Mutants. Among the processes a structures mentioned, i.e., initiation a differentiation of the original follic mitotic activity and structure of the bu keratinization of the inner sheath, ha and club, migration of shaft, and regene tion cycle, there are steps affected by m tant genes and by teratogenic agents. T genotype for crinkled (*cr/cr*) results in initiation of follicles in the 12½ to 17-d embryo and again in the postnatal peri (Falconer *et al.*, '50–'52). Consequent there are fewer sinus hairs, no monotr guard hairs, and no small zigzags, but the hairs are awls or hairs most nearly sembling awls. Furthermore, there is faulty differentiation of follicles, some ing abortive and others producing ha

ith an irregular number and arrange-
ent of medulla cells across the hair. The
llicle seems to be defective in both its
rigin and the supply of cells delivered
om the bulb to the shaft. It is not clear
at rate of growth (i.e., emergence of
aft) is reduced; therefore migration may
e normal, and certainly the cycle, as dis-
layed by the catagen changes, is not
odified. A mimic of crinkled is the sex-
nked Tabby (*Ta*), a semidominant in the
emale (Falconer, '53).

In the fuzzy phenotype (*fz/fz*) the pel-
ge was thought to consist only of zigzags
f abnormal appearance (Dickie and Wool-
y, '50), but Silvers and Lane ('58) in a
oss with crinkled have shown that the
airs are presumably very irregular awls
r auchenes as well as abnormal zigzags.
n any case, there is a reduction in the
itiation of follicles, and a thin hair ir-
gular in medulla cells and irregularly
urved is produced. Although slow in ap-
earance, there is no evidence that rate
f emergence of the shaft is reduced.
xcept for having thinner hairs and the
obable presence of all the basic hair
pes, this case is much like the crinkled
utant in the irregularities of the hairs
emselves.

The wuzzy rabbit (Crary and Sawin,
9) may fit into this category with
inkled and fuzzy by virtue of its gross
ppearance, but the defective cuticle and
creased sebaceous secretion causing
umping of hairs suggest that the cate-
ory of keratinization defects would be
ore appropriate. Furthermore, the types
nd general regularity of structure of the
airs are basically normal, completely un-
ke crinkled, fuzzy, and Tabby in the
ouse.

In Ragged heterozygotes (*Ra+*) there
e some follicles that never differentiate
produce hairs, probably mostly poten-
al zigzags. The orientation of follicles is
mewhat abnormal with respect to each
her and with respect to the angle with
e epidermis (Slee, '57). Slee also points
t that follicles are often not in phase
ith each other in the growth cycles and
e normal fluctuations of skin thickness
ith hair cycles (Chase *et al.*, '53) do not
cur. The abnormal orientation and cur-
ature of follicles in anagen are often re-
tained in catagen and telogen. In the homozygotes (*Ra/Ra*), the condition is more extreme, but most interesting is the excessive thickness of the epidermis in early anagen. In normal mice this thickening occurs transiently (Chase *et al.*, '53) then decreases drastically as the mitoses in the bulb compete with the germinal epidermis. Slee ('57) suggests that the epidermis in Ragged remains thick for a longer time and even increases its hyperplasia because too few follicles come into full production to compete effectively.

Naked is a semidominant (*N−*) in which there is a fault in the keratinization of the hair; the homozygotes have hairs that break off at the surface as they emerge and also have some coiled hairs unable to emerge, but the heterozygotes have hairs that break only at weak points after emergence. The cuticle of the hair is often defective (David, '32; Steinberg and Fraser, '46). There is no effect on initiation of follicle, on hair cycles, or on migration rate, but there is a pronounced abnormality in keratinization.

A mutant type known as hairless (*hr/hr*), as well as its rhino (hr^{rh}) and bald (hr^{ba}) alleles (Fraser, '46; Chase and Montagna, '52; Montagna *et al.*, '52; Garber, '52) causes a defect in the hair cycle. The follicle fails to shorten properly at catagen resulting in fragments that later become cystic. The anchoring club also fails to keratinize, and the hair falls out as the shaft migration continues after mitoses cease in the bulb. Only monotrich follicles in hairless mice survive and produce hairs in the next hair generation, and even these become fewer in later generations (Mann, unpublished). A somewhat similar but less-drastic mutant is furless, *fs/fs* (Green, '54). Tufted (*tf/tf*) probably is a defect only in the club formation, the hairs falling out at each telogen but all being replaced, resulting in a changing tufted appearance (Lyon, '56).

Another defect in connection with the hair cycle is angora, a mutant not yet observed in the mouse but found in the guinea pig and in the rabbit (Crary and Sawin, '53). The anagen phase is abnormally long with a very short telogen as in

some sheep wool fibers and in human scalp hairs (cf. Chase, '54, '55).

Of the various mutants that cause wavy hair, little is known about how the curvature is produced. Crimping as in the normal zigzag hair is probably the result of a narrow shaft and an unequal deposition of keratin. The kinks alternate and may be related to the direction of the bend immediately above the bulb that "rotates" during anagen (Auber, '52, in sheep). A curved hair may be the result of less drastic but more continuous differential growth or amount of keratin on the two sides of a hair shaft. Another explanation may be that the hair follicle and canal are curved. In Rex (*Rx*—) there are comparable curvatures that persist through catagen and telogen (Chase, unpublished), suggesting that this is a cause rather than a secondary effect.

Aside from the waviness of hair, there is the question of hair slope, best displayed by mutant genes in the guinea pig influencing the character Rough (see Wright, '34, '50 for references and discussion). Presumably, differential growth among the layers of the skin is generally responsible.

A new variant in the C57BL strain, which may represent a primary defect in the migration process only, is being investigated. There is a coiling and wavy condition of the hair, which appears as if cells are being supplied faster than the hair can emerge. Some hairs eventually emerge in normal condition, others do not. This situation is mentioned here, although little studied yet, because it may represent a true migration failure or a lack of synchrony with the cells produced by the bulb.

Teratogens. Although the term, teratogen may not be used here in the conventional sense (i.e., agents acting on embryonic development), nevertheless these agents do produce effects on developmental processes and are consequently called teratogens. The agents to be mentioned are those of biotin deficiency, of X irradiation, of colchicine treatment, and of mechanical wounding. Biotin-deficient mice (Rauch, '49) are defective in the keratinization process, but have normal differentiation and hair regeneration cycles. The effect simulates that of Naked. High doses of locally applied radiation to newborn mice can, as mig be expected, cause a failure of norm differentiation of new follicles. A hi dose (of the order of 3000 rads) can cau some already active follicles to fail shorten and can cause them to atrophy, in the hairless mutant. This, too, is result not unexpected in view of t amount of skin damage. A moderate do (400–700 rads) however, can bring abo temporary epilation by causing the acti bulb to become disorganized, even thou many cells continue to undergo mitos for a day or more after an initial cess tion of only a few hours. Rate of ha migration is reduced but continues. Sin no normal club is formed and the ha continues to emerge, the hair falls out 4 days. The keratinized terminal filu formed the day after irradiation is oft wavy, indicating faulty keratin or a f mation faster than the shaft is emergi or both.

Colchicine reduces cell proliferation the matrix of the bulb of the anagen ha follicle (Malkinson and Lynfield, '59), sulting in the production of a narrow shaft, again supporting the concept th migration of shaft is comparatively inc pendent of the supply of cells from t bulb. When the treatment is more seve the hairs break off at the narrowed regio As regards the disturbance resulting fro a mechanical wound, some follicles at t healing edge become curved and produ curved hairs and others have a change slope, but no changes as to type of ha produced have been noted.

PIGMENT

General description. In pigmentatio several processes and structures can be fected; all, however, relate directly or directly to the melanocytes. The stem ce of neural crest origin (Rawles, '40, '4 are incorporated in the follicle as it d velops from the basal layer of the e dermis. The stem cells of immediate co sequence are those that come to lie in t upper bulb region in contact with t dermal papilla (Chase *et al.*, '51; Cha '54). Certain phenotypes differ in t number, location, and persistence of the cells.

Mature melanocytes develop from the em cells and may migrate in some cases om the dermal papilla. They supply the elanin granules to the recipient potential air cells that pass outward from the bulb become keratinized as the hair shaft. ormally, a mature melanocyte lasts rough one hair generation, then regresses nd is lost. Granules within the melano- yte exhibit variations in size, shape, and umber associated with various phenotypes Russell, '49). Furthermore, clumping of anules alone can result in a different henotype, for example, that of dilute. Ielanin is laid down on the granules vary- ng in amount, duration, and quality.

Mutants. Only a few mutant examples ill be presented, but they will serve to ndicate some of the variations that may ccur. In piebald spotting (*s/s*) there is pparently a lack of stem cells in certain egions of the skin. At present it is not ossible to determine if the lack is the re- lt of a neural crest failure or merely the ailure of stem cells to develop into mature istinguishable melanocytes. In silver *si/si*), there appears to be a deficiency in ie number of stem cells, but again the eficiency may be a failure of some of iem to develop into mature melanocytes. ne variation of silver in the mouse, but ot true for all alleles at this locus or all ackgrounds, is a failure of the melano- ytes to continue melanogenesis through- ut the growth of the hair, resulting in pale to white base of the hair. In this onnection the Light mutant, B^{lt}/B^{lt}, $B^{lt}/+$ Quevedo and Chase, '58), has a melano- yte that also fails early; in some cases it ecomes detached and even incorporated nto the hair shaft. Dilute (*d/d*), with its umped granules, is a phenotype in which ome granules are taken into the recipient ells in the ordinary way, but the clumps re usually carried out between medulla ells. On occasion the whole melanocyte ay be carried along as in the previously entioned Light phenotype. Albinism is situation in which there are mature elanocytes in normal position (Silvers nd Russell, '55) producing and delivering anules normally, but melanin is not de- osited on the granules. Other alleles at is locus produce more or less melanin and also do not affect the granules or melanocytes directly.

Yellow (A^y—) has a different quality of melanin on fewer than normal granules, but is of particular interest because it is the hair follicle that determines this behavior of the pigment cell (Silvers and Russell, '55). In contrast, dilute has a different pigment cell not only in the hair follicle but also in the skin. The Agouti hair has not yet been fully explained and is also of particular interest since melanocytes change their melanin production for a short time only, during the hair growth, resulting in a subterminal yellow band on a dark hair. Monotrichs and vibrissae are not banded.

Some granules are modified in the recipient hair cells, the basic granule being small and round as in brown (*b/b*), elongate in Black (*B*—), and small and shred-like in pink-eyed dilute (*p/p*).

Although mutant genes are known that influence the distribution of stem cells, the persistence of mature melanocytes, the type of granule, the clumping of granules, and the melanization of granules, there is no mutant that otherwise directly influences the cytocrine capacity for transmitting granules into recipient cells.

Teratogens. Studies of this type add a little information but not as much as might be expected. Skin transplants, not usually considered teratogens, indicate the dependence of pigment cells on hair type (Silvers, '58a, b), and also reaffirm the neural crest origin (Rawles, '40, '47). Biotin deficiency (Rauch, '49) causes a reduction in melanization that can be restored in 24 hours because the active melanocytes with granules are still present and functional, lacking only normal melanin deposition. X irradiation, although temporarily and abortively increasing melanogenesis of mature melanocytes, has as its main action the destruction or inactivation of stem cells (Chase, '49), causing graying in subsequent hair generations. In some cases one or two melanocytes do occur after irradiation, giving a hair that is only partially pigmented, i.e., a mosaic. In other rare cases, mature melanocytes are present in the dermal papilla in the hair generation after irradiation and seem unable to enter the epithelial cells of the bulb and thus

deliver granules. Either stem cells have been ejected into the papilla, or the cells enter normally from the papilla and have been hitherto undetected because they enter before becoming melanogenic (cf. study on the fowl, Foulks, '43; Espinasse, '59).

There are other agents that affect the loss of pigment in the hairs by inactivation, destruction, or even by preventing melanization. There is, however, no outside agent yet known that converts, for example, brown to Black or non-Agouti to Agouti.

SUMMARY

Mammals lend themselves particularly well to phenogenetic studies, currently at least, at the level of developmental and physiological mechanisms and eventually more at the biochemical level. Examples of pleiotropism, or spurious pleiotropism, are most often investigated under the heading of phenogenetics and have yielded much information on the nature of gene action at the level of developmental sequences and inductions.

In this paper, however, interesting pleiotropic effects are ignored as much as possible in order to place emphasis on the basic mechanisms and structures at which mutant and teratogenic effects are observed, although obviously there are underlying mechanisms and biochemical lesions not yet resolved. A scheme organizing these basic mechanisms and structures into a few broad categories relating to hair and pigment production is presented along with selected pertinent examples.

OPEN DISCUSSION

WRIGHT[2]: Do you have any direct evidence on whether the same pigment cells that produce black pigment shift to production of yellow and back in the development of Agouti hair, or whether there is replacement by other pigment cells from one phase to the other?

CHASE: I think it is the same cell in the Agouti and that, at one time in the hair cycle, melanocytes change from black to yellow and back to dark. The evidence at present from my point of view it that it is exactly the same cell and that it simply changes, but I don't know how.

MARKERT[3]: Have you ever seen a c with both yellow and black granules in

CHASE: I think so, but rarely. With u stained slides and under phase contr only, I have seen what appear to be da granules at the end of a dendrite, yell granules in the karocyte, never the oth way around.

MARKERT: You probably have look at more cells than I have, but I have nev seen a cell containing melanin granules two different colors.

HOLLANDER[4]: I simply wanted to qu tion the logic in that last slide where y have phenogenetic interpretations. Y had, for example, homozygous *p* and al *P* concerned with the same action. seems to me that the logic here is getti scrambled if we use the wild-type allele one place and not in another. You did n use *B* along with *b*.

CHASE: Calling a wild-type allele a m tant is obviously poor terminology, but did want to include Agouti. However, t reason I have the brown and black is th there is a difference, as you know, in t type and shape of granules and in t melanization.

SILVERS[5]: I agree with Dr. Chase th in the Agouti pattern, one melanocyte c produce both black and yellow pigmen In my experience I have not been able see both types of granules in one ce However, I think this is because the chan between eumelanin and pheomelanin such a gradual one that there are inte mediate stages. Dr. Chase, since you thi that one cell can produce both types pigment, are you willing to go one st further and say that the next hair gener tions are supplied from the mitotic d scendants of previous generations? Or you think that all melanocytes of each ha generation "shoot their wad," so to spea and must be replaced from a stem ce reservoir?

CHASE: I now think the latter. I kno that there was a time when I thought t other way, but I now think that they "shoot their wad." Finally, from the e

[2] Sewall Wright, University of Wisconsin.

[3] C. L. Markert, Johns Hopkins University.

[4] W. F. Hollander, Iowa State University.

[5] W. K. Silvers, The Wistar Institute of An omy and Biology.

ence on the Light mutant and then look-
ng more carefully at the very last pig-
ientation at the end of the hair cycle in
ie normal situation, I think they actually
re gone. They come again from the stem
ells, but not from the active melanocyte.
o I no longer agree with my old scheme
f having melanocytes go through a dor-
iant phase and back again.

MARKERT: You said that spotting might
e interpreted as caused by an insufficient
upply of stem cells. Yet in many spotted
nimals, many of the hairs are densely
igmented. Does this indicate an insuffi-
ient supply?

CHASE: In certain areas there are hair
ollicles that do not get a supply of these.
et, in another area there are plenty, as
there is a defect in the differentiation of
ie neural crest. In this defective area
iere would be no pigment cells produced.

WRIGHT: The spotting factor *s* of the
uinea pig has more-extensive effects than
ierely causing a pattern of color and
hite. The tortoise shell pattern (gene
) is black with occasional yellow hairs.
i $sse^{v}e^{v}$, the colored areas become divided
ito black and yellow spots in which the
iapes of the latter simulate and extend
hite areas. Also in dingy browns
(*EbbCCPPFF*) the distribution of dingi-
ess is changed from a smoothly graded
attern to one of spots in which dark and
ght dingy areas are related to the white
reas. Thus the boundary between dark
nd light is often continued by a white
reak, showing that the spotting of dingy
rown is related to the process that deter-
iines the distribution of color and white.
i rare cases, the spotting gene *s* has an
ffect on certain other colors in addition
o its effects on patterns of color and white,
f black and yellow, and of grades of
inginess.

MARKERT: Would you place the re-
ponsibility for spotting on the hair follicle
the general environment of the melano-
yte, rather than on the melanocyte itself?

WRIGHT: Primary gene action may be
ther in the melanocyte or in its environ-
ent, I am not sure which it is. One of
ie end results is a failure of the pigment
ells in the areas that become white. My
oint is that there are spotting effects of
less drastic sort with respect to certain
color processes within the areas in which
melanocytes persist.

CHASE: Actually, the possibility here is
that, instead of a failure of the stem cells,
there is a failure of these cells to produce
a mature functioning active melanocyte.

WRIGHT: In animals that are hetero-
zygous for almost any factor, one occa-
sionally (say, one in 5000) gets what looks
like a somatic mutation, a spot of the
recessive color. Most of the observed cases
have been in white spotted animals (*s/s*).
The recessive color is related to white areas
in the same way that yellow spots are in
tortoise shells. Dunn has noted a similar
situation in mice.

CHASE: Actually Mr. Schreiber (Iowa)
is working on this sort of thing.

E. S. RUSSELL[6]: It seems to me that loss
of stem cells is a thing that could easily
happen in a variety of ways in different
spotted genotypes, so that many things
could account for a loss.

I would like to comment on the very
frequent association, in the mouse at least,
of spotting genes with other very deleter-
ious effects, so that things that kill off
melanocytes or their stem cells must also
be very deleterious to some other kinds
of tissue. A thing that might be interest-
ing to consider is something first dis-
covered, I believe, by Dr. Dunn. If you
combine A^{y}/a, in place of a/a, with a large
number of different spotted genotypes, you
increase the colored areas and decrease
the white areas of the pelage. This sug-
gests some relation between the type of
pigment to be formed and the possibility of
the melanocyte or stem cell surviving.

STRONG[7]: Dr. Chase, how do you ex-
plain the aging changes in pigmentation?
For instance, a black sometimes becomes
mahogany or rusty colored.

CHASE: In some black pigment, as it
gets old, there is a change to a brownish
color. If you mean that a new hair that is
formed with a new set of pigment is
mahogany, I don't know.

MARKERT: Why don't they become
gray?

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[6] E. S. Russell, Roscoe B. Jackson Memorial Laboratory.

[7] L. C. Strong, Roswell Park Memorial Institute.

CHASE: Some do, and that is a loss of stem cells or a loss of the capacity of the stem cells to become mature melanocytes.

WAELSCH[8]: I wonder what the situation is with respect to the stem cells in *W*. They must have been looked at. Are melanocytes found in the all-white?

SILVERS: As far as the homozygous *WW* is concerned, which is completely white with black eyes, there seems to be no recognizable amelanotic melanocytes as *do* occur in the albino. The black eyes are the result of retinal melanin but the choroid is completely devoid of pigment. For this reason, it is hard for me to believe that these all-white animals are any different from the white spot that occurs on a pigmented animal. Indeed, it is best to think of these phenotypes as being "one big spot." Dr. Clement Markert and I have attempted to determine whether white spotting is the result of an environmental arrest in melanoblast differentiation by explanting neural crest containing tissue from animals destined to be completely white (i.e., "one big spot") into an environment, the anterior chamber of the eye, that is known to be favorable for melanocyte differentiation and pigment production. Our reasoning was that, if in such an environment our explants became pigmented, the inability for pigment formation in the mutant(s) could be attributed to an environmental effect. In no instance did we ever find any pigment cells in these explants. However, since neural crest cells from potentially pigmented genotypes sometimes also failed to produce pigment in the graft to the eye, our results may not be as meaningful as they seem.

MARKERT: I would like to supplement Dr. Silvers' comments very briefly. A couple of years ago, Dr. Strong was kind enough to send me a recessive black-eyed white mutant variety of the C3H strain. We made a fairly extensive series of tests with these mice by the same techniques Dr. Silvers just described, namely, by explanting the neural crest of embryos to the eye of mice in which we knew pigment cells could develop. In no instance did we ever obtain any pigment cells from these black-eyed white mice. These results tend to corroborate the hypothesis that the deficiency in the homozygous W animals a in this black-eyed white strain of Strong's lies in the failure of the neu crest to differentiate a cell that is capa of becoming a melanocyte. This would a true deficiency in the stem cell.

WAELSCH: Did the stem cell its spread?

MARKERT: We would have no evide that there is any stem cell at all.

WAELSCH: An extreme example of su a condition is of course the Splotch r tation in mice; there, the homozygotes at about 14 or 15 days of gestation. T embryos have a wide open neural tube, a migration of the neural crest cells may inhibited mechanically. Explants fr such embryos do not form any pigm except for the retinal pigment in the e

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Mammalian Pachytene Chromosome Mapping and Somatic Chromosome Identification[1]

A. B. GRIFFEN
The Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

In an initial study of the germinal chromosomes of the mouse (Griffen, '55), a provisional pachytene map was constructed for use in the cytological identification of the genetic linkage groups. This map, prepared with the use of Zeiss (Jena) apochromatic objectives, provided an accurate approach to the chromomeric organization of the chromosomes, and the use of oblique transmitted illumination made possible the delineation of many of the finer details that might otherwise escape detection.

When the map was completed, it was recognized as having many defects, some of which might be corrected in the course of its use in chromosome identifications. These defects have indeed proved to be abundant. Perhaps the most outstanding fault was the absence of properly identified centromeres, without which the chromosomes usually have no dependable reference points from which chromomere counts and comparisons can be made. A fault of more fundamental nature was that the map was made with full-resolution microscopy and thus contained details that are regularly observed only in nuclei that approach the ideal in staining, chromosome spreading, and flatness. For the vast majority of nuclei, which lack one or more of these simple qualifications, the map was too complex for ready use; and for the nuclei of tumor cells, which are easily studied only with phase-contrast microscopy, the map proved almost useless. Accordingly, the revision of the map was planned as a phase-contrast project, in which each chromosome should have its centromere position clearly indicated. This map, though more easily used than the original, must still be considered a provisional and preliminary reference work (fig. 1).

MATERIALS AND METHODS

General procedures. Male mice from strains C57BL/10 and C57BL/6 were the source of material. The testes were removed from animals that were killed by cervical dislocation at the age of 28–40 days. Each testis was immersed in the stain fixer for dissection. With needles and forceps, the tunica was torn open and the tubules were separated and loosened in order to assure rapid and even penetration. For each slide, several long pieces of tubules were transferred to the slide and covered with a fresh drop of the stain fixer. These fragments were then finely minced with needles, covered with a No. 1 cover glass, and blotted with filter paper. Under a dissecting microscope the cover was pressed with needles to break the tubules, free the cells, and rupture the nuclear membranes.

Each slide was examined under the compound microscope for sharpness of staining and suitability of spreading. Satisfactory slides were dehydrated by the alcohol vapor method (Bridges, '35) for 12 hours, immersed in 95% alcohol for 1 hour, and made into permanent mounts with Diaphane.

Reagents and timing. Throughout the study the only stain-fixer used was a Sudan Black B solution in lactic, propionic, and formic acids, prepared according to a formula developed by Cohen ('49). A brief outline and discussion of the Sudan Black method in mouse cytogenetics has been given previously (Griffen, '55). The

[1] This work has been carried out under Research Grant C-1912 from the National Cancer Institute of the National Institutes of Health, Department of Health, Education, and Welfare; a grant from the Elsa U. Pardee Foundation; and Grant AT(30-1) 2113 from the United States Atomic Energy Commission.

staining time varied from 30 to 120 minutes, with 45 to 60 minutes seeming to produce the optimum degree of contrast and differentiation. The differentiation is similar to that produced by the Feulgen reaction, but in tones of brown and black rather than red.

Microscopy and drawings

A Zeiss-Winkel GF microscope with an integral illuminator was used for all studies, equipped with Neofluor phase-contrast objectives and compensating oculars 8, 16 and 20×. The 20× oculars were used only in connection with a Zeiss-Winkel tubular drawing attachment, whose operation is similar to that of a camera lucida. No filters of any type were used.

Preliminary outlines and all possible d tails of chromomeric structure, as well the size and position of the centromer were obtained with the drawing apparat All fine details and all decisions as to t appearance and organization of chrom meres were completed after the drawi attachment was removed. A minimum five original drawings was used in t construction of each map element.

Centromere locations

In order to determine the appearan and location of the centromeres, wh identity in several of the pachytene ch mosomes had been indicated tentatively the first map (Griffen, '55) a study centromere locations in *Drosophila* beg in collaboration with Dr. Mary Wart

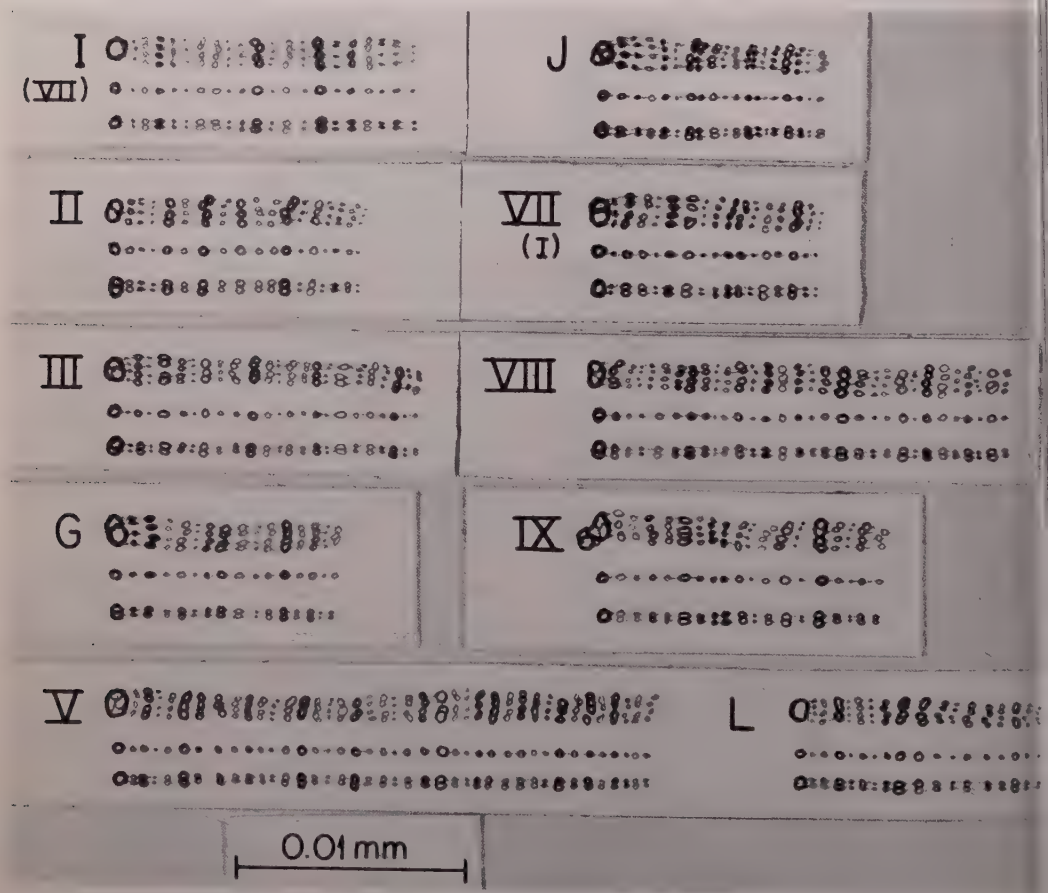

Fig. 1 Pachytene chromsomes of the male mouse.

as resumed and completed. In this investigation, which involved extensive irradiation experiments, it was shown that ne centromeres in interphase salivary land cells are heavily invested with nucleic acids and therefore appear as densely tained bodies; and that the centromeres, eing homologous and nonspecific, regularly synapse to form dense clusters from hich the chromosome arms extend (Wars and Griffen, '59; Griffen, '58).

Upon careful search and study of mouse achytene cells, it was found that each ucleus contains several centers, each reembling the single centromere cluster of *Drosophila* cell. From each center exend two or more chromosomes; the greatst number observed was seven, but the ost common numbers are three, four, and five. There is no indication that all of the centers may have been clustered into a single body before slide preparations, since small unruptured nuclei readily reveal several centers, widely separated and clearly independent of each other. There also has been no indication that particular chromosomes tend to be associated in any of the centers. On the contrary, membership in the centers seems to be entirely random. There is no reason to doubt that these prominent synapsed bodies, which are strictly comparable with those demonstrated in *Drosophila,* are the centromeres.

All use of the terms "heterochromatin" and "heterochromatic" is strictly avoided in reference to these centers. The heterochromatin concept has brought certain profitable lines of investigation to an

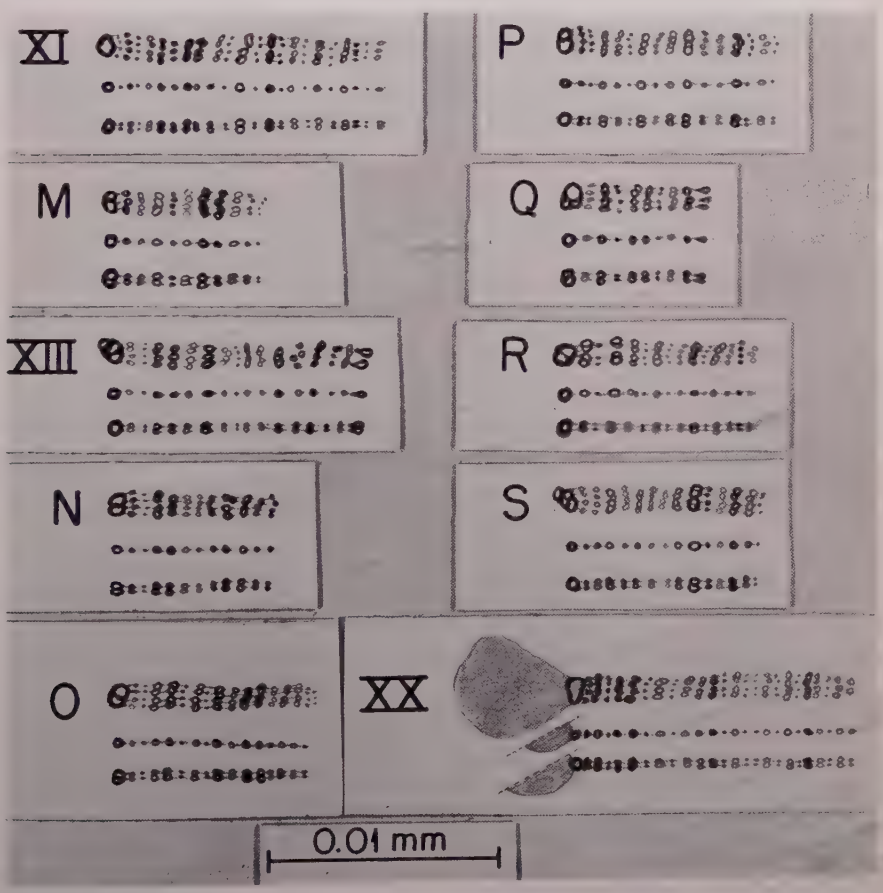

Figure 1 (*Continued*)

abrupt halt in *Drosophila*, where the term "heterochromatin" actually serves as a cytogenetic wastebasket into which is thrown almost every cytogenetic problem that has no immediate and obvious solution. It is most important that this concept shall not be carelessly introduced into mammalian cytogenetics, where it can do extensive harm and bring discouragement to investigators whose tasks are already most difficult.

As germinal chromosome studies continue, particular attention will be directed toward the apparent random association of chromosomes in the several centromere clusters. Any evidence of nonrandom associations will be of considerable interest in that it may help in clarifying the problems of "association systems" as suggested by Gates ('26) and the "affinity" phenomenon as presented by Michie ('53) and Wallace ('53).

The phase-contrast map

In accordance with these findings, it was determined that all chromosomes intended for use in the construction of the composite map should have their centromeres intact; for these bodies serve as a dependable beginning point for each element, and make identifications possible even when distal portions of the chromosomes may be missing through mechanical injuries associated with slide preparation. In the map, each element is shown with its centromeres on the left. When it is necessary to identify a specific chromomere in any chromosome, the count begins with the centromere as chromomere one, and proceeds to the right in simple numerical order, without any subdivisions or other arbitrary demarcations.

Nomenclature. The system established in the 1955 map, that of providing Roman numerals for elements that have been identified with linkage groups, and noncommittal letters for elements not so identified, has been continued. The excellent studies of Slizynski ('57) on the translocations analyzed by Carter, Lyon, and Phillips ('55) have given pachytene identities to linkage groups, I, II, III, VII, IX, XI, and XIII, and have confirmed the previously determined V and VIII. Since the conclusive identification of groups I and VII must await the production of an additional translocation involving either these chromosomes, the designations I and VII must be considered tentative. The numerals in parentheses to the left of the elements in the phase map indicate the possible need for exchange of the designations. Both Slizynski and I agree on the nucleolar chromosome as the XY tetrad; neither has as yet published studies on a sex-linked translocation, but several presumed examples, marked by the X-chromosome gene Bent-tail, are expected to be available. Slizynski's identifications ('49) based on his 1949 map and my 1955 map are shown in table 1; I concur fully in these identifications.

TABLE 1

Pachytene identities of linkage groups

Slizynski's map element	Griffen's ('55) map element	Linkage group
13	A	VII
15	B	XI
9	C	II
14	D	III
17	E	V
11	F	I
18	H	VIII
16	I	IX
19	K	XIII
20	XY	XX

Arrangement. Continuing the principles proposed in the 1955 map, chromosomes A, B, C, D, F, I, and K have been given their linkage group numbers and placed in the appropriate positions on the phase map. Below each pachytene chromosome two drawings are added. The first shows the expected appearance of a single chromonema, such as might be found in an early prophase nucleus. The second shows the expected appearance of a pair of chromonemata, such as might be expected in a later prophase nucleus. At this time, these two-stranded figures are being used with encouraging success in the identification of chromosomes in the late prophase stage of tumor cells.

Figure 2 is a prophase cell from an ascitic growth of an ovarian teratoma, whose nature has been described elsewhere (Griffen, '58). Tentative chromosome identifications in this and in other prophase tumor cells were made on carefully constructed

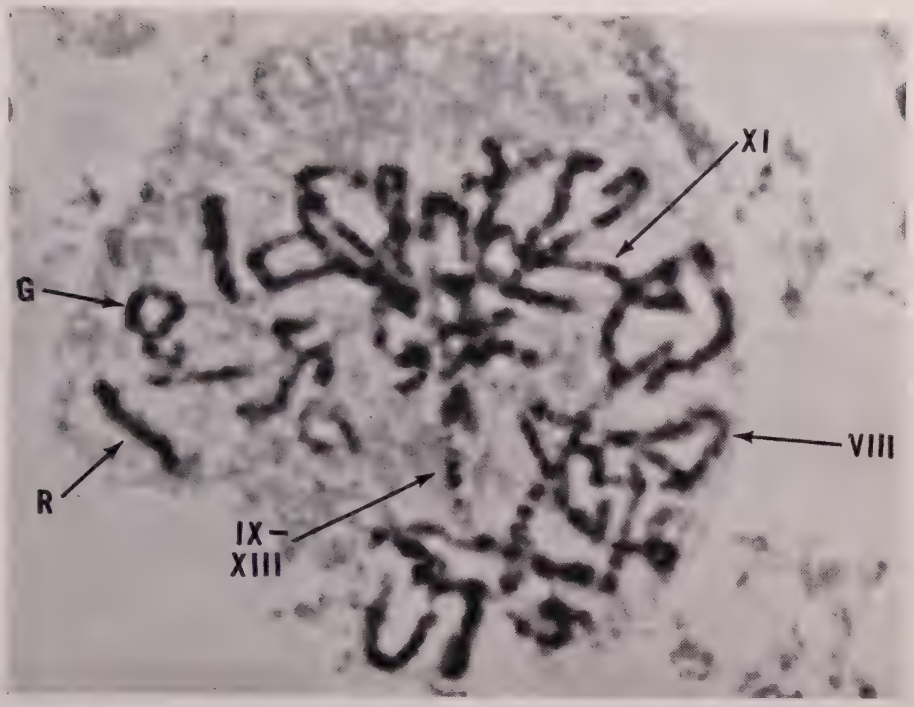

Figure 2

rawings, which were compared with the ıap. The arrows indicate several of the ements that have been identified, includ- g one that is a compound of chromo- mes IX and XIII and is presumably the sult of translocation.

DISCUSSION

The phase-contrast map omits numer- ıs details that could be discerned with é use of apochromatic lenses and trans- itted oblique illumination, for phase icroscopy provides contrast at the ex- ense of resolution. Table 2 compares the ımbers of chromomeres in each chromo- me of the 1955 map with the numbers own in the corresponding elements of e phase map.

The total count of chromomeres in the ll-resolution map is 471, and in the ıase-contrast map is 384. This reduction the amount of detail can easily be re- rded as a step in the wrong direction, for assic cytogenetics usually seeks to dis- ver and utilize ultimate structure, taxing e capacity of the finest microscopy. In presenting the phase map, I by no means abandon the classic ideals but rather seek to provide a guide that may permit germinal cytogenetics and tumor cytogenetics to find a common meeting ground, and that at the same time has a reasonable degree of simplicity. This latter quality should be of value to seasoned investigators in mouse cytogenetics, as well as to newcomers in the field.

Since the centromeres are indispensable guides in the use of the map for chromosome identifications, many of their varied appearances are illustrated. Chromosomes II, M, N, O, and P show two centromeres for each tetrad, which is considered to be the fundamental organization. The remaining chromosomes show parts of centromere clusters, which may appear as large hollow spheres, morulae, or short rows. In any cluster illustrated, only two of the centromeres belong to the attached tetrad. The large spheres, as in I and L, may be called "phase artifacts," since they appear as loose morulae when the sub-

TABLE 2

Comparison of chromomere numbers in the full-resolution (1955) and phase-contrast (1959) maps

1955		1959	
Chromosome	Chromomeres	Chromosome	Chromomeres
F	19	1	21
C	20	2	18
D	24	3	24
G	19	G	16
5	43	5	40
J	18	J	19
A	29	7	17
8	35	8	31
I	29	9	19
L	22	L	18
B	26	11	23
M	15	M	11
K	28	13	17
N	16	N	12
O	24	O	17
P	21	P	16
Q	13	Q	10
R	22	R	15
S	14	S	15
XY	34	XX	25

stage phase plate is removed to permit normal resolution.

All chromosomes in the map are shown as telocentrics, with the possible exception of the XY in which the plasmosome may represent a small second arm. Although small "left" arms have never been observed, they may (and probably do) exist; the occurrence of persistent ring-chromosomes in certain tumor cells (Griffen, '58) strongly indicates that some chromosomes, at least, have second arms that are long enough to permit frequent involvement in aberrations.

SUMMARY

Through the use of Sudan Black B staining and phase-contrast microscopy, a simplified pachytene chromosome map has been prepared. Ten of the possible twenty linkage groups have been identified on the basis of Slizynski's translocation studies. Each pachytene tetrad possesses two centromeres, which serve as indispensable landmarks in chromosome recognition and mapping. As in *Drosophila*, the centromeres of all chromosomes are homologous and nonspecific, as indicated by their apparent random association in the form of loose clusters. The phase map makes possible the identification of chromosomes in favorable mitotic prophase stages of certain tumor cells.

ACKNOWLEDGMENTS

Without the friendly interest and help of his colleagues, perhaps no one would be willing to undertake, for a second time, the preparation of a mammalian chromosome map. I am indebted to all my colleagues for their interest, and particularly to Dr. E. Fekete, Dr. G. D. Snell, Dr. E. J. Murphy, Dr. E. L. Green, and Dr. W. J. Murray for their continued encouragement; and to Mr. Merrill C. Bunker, who shared in the preparation and initial study of most of the material.

OPEN DISCUSSION

FORD[2]: I should like to comment on the stand Dr. Griffen has taken against the use of the word "heterochromatin." He isn't the first to object to it, nor am I. There was a letter in NATURE by Baker and Callan ('50) pointing out that there were many differentiated regions of the chromosomes and that to call them heterochromatin and then suppose they all had exactly the same properties was a delusion and a snare. They suggested that the word

[2] C. E. Ford, Radiobiological Research Unit, Harwell, Berkshire.

eterochromatic"—the adjective—could used and used safely to connote differtial behavior. But we get into quite a ess by supposing that what is commonly lled heterochromatin in one species or ie chromosome will behave in precisely e same way as apparently similar reons in another species or another chroosome. It is obvious that, in detailed udies of the chromosomes of any species, ere are these differentiated regions, but at they do not always react in precisely e same way.

GRIFFEN: There is no point with which disagree.

KAPLAN[3]: I like the Zeiss-Winkel microope. I also like heterochromatin. I beve that what is observed at the centroeric region of every mouse chromosome, very rat chromosome, and every opossum romosome that we have studied is heterhromatic material. In addition there is block of heterochromatin at the distal nd of each chromosome and smaller eterochromatic regions variously located, ut characteristic for each chromosome. iese regions are metabolically out of ase with the other regions of the chroosomes, and during interphase and early ophase they are more deeply staining or sitively heteropyknotic.

The deeply staining bodies that Dr. Grifn talks about are surely heterochromatic gions of individual chromosomes and not e kinetochores themselves. Kinetochores e not usually visualized by ordinary ethods and when seen are in the form tiny granules less than 0.5 μ in diameter. erhaps I have not understood Dr. Griffen rrectly.

One prominent positively heteropyknotic dy that we observe in males during the ophase of meiosis I, through early diplone, is the sex vesicle, a nucleolus-like dy that constitutes one of the twenty valents that may be counted during chytene or diplotene. Prefixation treatent with ribonuclease or distilled water scloses the individualities of the chromomes contained within, the X and Y in d-to-end association. This sex vesicle is t seen in meiosis of the female mouse rat. The XX bivalent in females behaves actly as the autosomal bivalents, premably permitting crossing over to occur freely. The end-to-end association of the X and Y and this peculiar arrangement within the sex vesicle undoubtedly represent an isolating mechanism that holds the male-determining factors of the Y within that body. I do not know whether this prominent body in pachytene nuclei is what Dr. Griffen is referring to. If it is, it is surely not analogous with the chromocenter of the salivary gland cells of larval *Drosophila*.

FORD: I have also seen the same kind of pachytene chromosome that Dr. Kaplan described, and undoubtedly there are the same darker staining bodies near what I believe to be the centomeres. I will try and say more explicitly what I meant when I said that I did not like the word "heterochromatin." If one finds a chromosome region that stains more heavily in the prophase of mitosis or meiosis one cannot infer that, if a translocation arose with a break in that region, it would show genetically a variegated-mottled effect; one cannot infer that it would necessarily show what Slizynski has called "ectopic pairing"; and one cannot infer that it would necessarily show the cold-induced "starvation" effect.

GRIFFEN: I wish to thank both Dr. Kaplan and Dr. Ford for contributing to that point. I have referred to heterochromatin rather pointedly and wish to make the plea, as I said before, that we do not use heterochromatin as a dumping ground for all sorts of things we otherwise cannot explain or that we want to leave obscure. In pachytene chromosome work that aims at honest mapping and strict linkage determinations, and in studies of tumor cells for the identification of chromosomes, it is not yet necessary to invoke heterochromatin.

STERN[4]: May I ask one more question? You showed us paired inversion and translocation configurations in what seemed to me somatic or at least nonmeiotic cells. Does that mean somatic pairing?

GRIFFEN: Yes. It is an actual synapsis. Perhaps this tumor has not forgotten that it started out to be an ovary. I cannot be more scientific about it than that.

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[3] W. D. Kaplan, City of Hope Medical Center.

[4] C. Stern, University of California, Berkeley.

CHU[5]: Though my experience with the mouse material is very limited, I would like to make a few remarks. First, I completely agree with Dr. Griffen on the importance of studying the pachytene chromosomes on the other hand, we should not neglect to compare and correlate the chromosomes at pachytene with those in gonial and somatic cells. The morphology of the mitotic metaphase chromosomes of the mouse is admittedly difficult, but it is possible to recognize at least four pairs, the Y and possibly the X chromosome. I now see the beginning of a comparison between the pachytene and the mitotic prophase chromosomes of normal cells, as Dr. Griffen has done with the tumor cells. The identification of individual chromosomes in somatic cells has important applications in solving problems such as cytological mapping of linkage groups and somatic mosaicism.

My second point concerns the position of centromeres in the mouse chromosomes. Tjio and Levan ('54), on the basis of their study on spermatogonial cells, mentioned that all mouse chromosomes are telocentric. Dr. Griffen's study on pachytene chromosomes seems to lead to the same conclusion. On the other hand, at least two pairs of somatic chromosomes seem to me to have short arms. May we then say that a few mouse chromosome pairs are acrocentric?

My third question is: How many nucleolus organizing regions are there in the mouse cell, particularly from your evidence in pachytene studies?

GRIFFEN: I concur with Dr. Chu in his remarks about the identification of somatic chromosomes and admire the beautiful work he has done on human somatic cells. It would be most useful to have somatic chromosome identifications in prophase stages, and I think it will be entirely possible. I do not believe I said that mouse chromosomes are telocentric. I pointed out that the map shows a centromere at the end because that is a good beginning point for each element. I would like to state clearly I do not think there can be a telocentric chromosome in any organism that I know of. I have yet to see something that is convincingly telocentric.

I still am an ardent proponent of t telomere idea. Beyond every centrom there must be at least a telomere, which the terminal unipolar body of the chrom some. I have not seen any short arms the mouse, and they are therefore not cluded on the map. But I shall alwa watch for them and include them wh ever I am able to find them. I hope oth will do the same.

You will recall that I mentioned ri chromosomes, which must have had ava able a short arm beyond the centromere the chromosome in which the ring formed. At least that would conform wi the picture in *Drosophila*, which again our refuge, you see, when we try to stu the smaller and more difficult bodies of t mouse.

I have consistently observed only o prominent and constant nucleolus organ ing region in male mouse pachyten namely, the one which is a part of the s bivalent.

WOLFF[6]: In reference to Dr. Stern question about the segmental interchan that was seen in a somatic cell, I think th was probably chromatid exchange a would not require synapsis in order to g the cross-like configuration.

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ıe Genetics of Vital Characters of the Guinea Pig

SEWALL WRIGHT
Department of Genetics,[1,2] University of Wisconson, Madison, Wisconsin

This discussion of vital characters of ɜ guinea pig will be divided into two ther diverse parts: first on unfavorable ɔrphological abnormalities and second quantitative variations in fecundity, ɔrtality rate, and growth. Discussion quantitative variations will be restricted gely to perinatal character.

The data are drawn from the records of colony of guinea pigs that traced to two ırces: an experiment on inbreeding in- ated in 1906 by G. M. Rommel, Chief of ɜ Animal Husbandry Division of the S. Bureau of Animal Industry, and nducted by the present author from 1915 1925 and a few animals that carried rious genes not present in the preceding, ıdly presented by Professor W. E. Castle 1916. The colony was almost wholly f-contained from 1916 to 1954. During ɜ period 1906 to 1925 some 31,000 in- ɜds were produced in 23 different strains aintained by brother-sister mating at the ltsville Laboratory of the Bureau of ımal Industry. About 5100 control ani- als were recorded up to 1925. This stock ıs derived from the foundation stock of ɜ inbred lines but was maintained with- t even second cousin mating. Some 4700 imals were derived from crosses between ɔred lines initiated after 1916. About 00 animals that traced largely to the ani- als presented by Professor Castle were corded in studies of factor interaction at ɜ Beltsville laboratory. Three of the in- ɜd lines were carried for a number of ars at the Whitman Laboratory of the ıiversity of Chicago after 1925 and pro- ced some 8000 young. The experiments factor interaction were greatly ex- nded and produced some 68,000 young. ıe total number recorded from the colony ıs thus over 120,000.

I. DELETERIOUS MORPHOLOGICAL DEVIATION

All observed morphological abnormalities were recorded and the genetics of certain types was studied intensively. The conclusions on the latter will be reviewed briefly. The implications of some statistical results will be considered in the case of those which were merely recorded. The abnormalities up to 1915 have been discussed previously (Wright, '22a). Most of the statistical results on abnormalities to be reported here relate to the 76,000 records at the Whitman Laboratory.

Otocephaly and cyclopia

Early inhibitions of the development of the head are among the characteristic types of abnormality in vertebrates. One type is absence of the mandible and ventral approximation of the ears (agnathia). Another is cyclopia. These may be combined, and in this guinea pig colony, cyclopia was never found without agnathia. There have been more than 500 guinea pigs recorded in the colony since 1906, which may be arranged with minor exceptions in a single linear sequence of 12 grades, collectively referred to as otocephaly. Mere shortness of the mandible (grade 1) grades into complete absence of the latter associated with a single median ear opening (grade 4, about 44% of all cases). In the higher grades these defects are complicated by defect of the upper jaw (5), defect of the forebrain (6), cyclopia (8) and in the highest grade (12) by complete absence of jaws, nose, eyes, all of the brain in front of the medulla, and almost all of the skull except petrosals and occi-

[1] Paper No. 776 from the Department of Genetics, University of Wisconsin.

[2] This investigation was aided by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago where the experimental work was conducted.

pitals (aprosopus). In this highest grade there is a normal body but no external indication of parts of the head except a single median diminutive ear with no opening (Wright and Eaton, '23; Wright and Wagner, '34—morphology; Wright, '34d—genetics).

The ordinary succession in which embryonic parts fall below the threshold of normal development is interpreted as (1) ventral mandibular arch, (2) frontonasal process, (3) maxillary processes, (4) olfactory placodes and cerebral vesicle, (5) median optic center of forebrain, (6) progressively posterior parts of the brain, (7) dorsal portions of mandibular arch, (8) hyoid arch. There have been occasional deviations from the usual sequence such as defect of the premaxillary without defect of the mandible (grade A, three cases) and presence of the maxillary below a cyclopian eye (grade 8A, 9A, two cases). All the defects visible at birth may be traced to defects of the neural crest (Platt, 1897; Stone, '29) or of the medullary plate and associated placodes and probably ultimately to inhibition of the prechordal mesoderm not later than an early medullary plate stage (Adelmann, '30).

Otocephaly has been rather uncommon in the general colony (about 0.04%) but a particular inbred strain (number 13) has produced varying percentages from none to 28% in large branches. Many have appeared among descendants of outcrosses to other strains.

The sporadic occurrence of monsters in a population suggests segregation either of a recessive gene or of a combination of genes at two or more loci. Both of these can be ruled out by the pattern of occurrence in branches of strain 13. Under brother-sister mating, the selection against a lethal condition, determined by one or more genes with full penetrance, would soon bring the proportion of monsters to 25% or more in those matings that produced it at all, even if dependent on multiple factors and soon thereafter would eliminate one of the necessary factors, from each line. The actual situation was wholly different. There was indeed segregation of different tendencies in the early generations among branches averaging 300 individuals. There were three branches with none, and four branches all with ab 1%. Four branches tracing to a sin mating in the thirteenth generation ied about 1.5%. The whole family u mately traced to another single mating the thirteenth generation. Of 8 branc of this, reported on in 1934, 7 avera 4.6% without significant deviations. O large group from a single mating in nineteenth generation was maintained about 28% for 15 years by selection fr high-producing matings. A large s branch relapsed to about 5%.

In all groups, from the sporadic cases the general colony to the high-produc branches of strain 13, the percentage affected females was approximately tw that of males.

All evidence indicates that, apart fr segregation of XX and XY, all individu in the branches producing 4 or 5% w homozygous for the same array of pert ent factors. There was very little m tendency for monsters to occur in the sa litters or from the same matings than pected by chance on the basis of a pr ability of about 0.06 for females and ab 0.03 for males. There was, however, slightly higher percentage incidence winter and early spring than in the r of the year and in smaller than lar litters.

The high percentage in most lines tr ing to the mating in the nineteenth gen ation rather clearly depended on the currence of a dominant mutation, t effect of which was to raise the probabil of abnormal development to about 0.20 a male and about 0.40 in a female. T probabilities were probably higher in hom zygotes but the evidence on this is n clear. All monsters above grade 1 died once after birth and those of grade 1 di soon unless the incisors were brok periodically to prevent the mouth fr being forced open. Two females of gra 1 from high-producing matings were ke alive in this way. Mated with a litterma and a half brother, respectively, they p duced no abnormals in a total of ni young, indicating as far as it goes that o cephalics have no excess tendency to tra mit the condition.

These monsters are similar to ones th have been produced by a great variety

vironmental agents (e.g., mechanical, emical, ultraviolet, cold) in experiments th fish (e.g., Stockard, '09), amphibia, d birds. Effective treatment may be as rly as the undivided egg but never after early medullary plate stage. The signifi- nt common factor seems to be acute par- l inhibition of the dominant region in e developing egg. It has been suggested at mammalian monsters of this type are oduced by maternal toxemia and that y hereditary tendency is maternal in ture. Experiments in the guinea pig ony definitely ruled this out in this case. e pertinent heredity was that of the onster itself equally derived from both rents (except for the X and Y chromo- nes).

It is probable that the action of the rtinent genes, though no doubt specific the metabolic level, is of the same non- ecific type at the morphological level as at of environmental factors, consisting erely of acute inhibition in some degree the dominant prechordal mesoderm. tual determination of abnormality de- nds on the conjunction of a hereditary ndency of this sort with unfavorable con- ions, pertaining in the main to that par- ular egg, rather than to all eggs in the me uterus. Otocephaly is a threshold enomenon, and the difference among verse genotypes is manifested merely by ferences in the percentage that fall be- v the homeostatic threshold.

Cruciate double monsters

It has often been shown that acute atment of eggs with injurious agents nds to produce double monsters in addi- n to ones with inhibited heads. Three uble monsters have been recorded in e guinea pig colony. All were of the uciate type, attached at the chests and th head or heads at right angles to the dies. One had two heads attached back back. One face was normal, the other cephalic of grade 6 (single median ear ening, median nostril, always a sign of divided cerebral hemispheres, but two es). Each of the other two monsters had ly one head with face of intermediate cephalic types (grades 5 with paired strils and grade 6). Two had ancestry strain 13 on both sides, the other none. Thus heredity may have played some role. The association of otocephaly with the doubleness probably reflects a severe inhibition of short duration at the very beginning of development by something unfavorable in the environment on a genetically susceptible egg.

Anotia

Inbred strain number 2 has never produced any otocephalic monsters in some 9000 young. Seven monsters of a superficially somewhat related type appeared, however, in a cluster of 160 animals descended from a single mating in the nineteenth generation of brother-sister mating in the portion of the colony maintained at Beltsville after 1925 under the supervision of Mr. O. N. Eaton (see Wright and Wagner, '34). These monsters resembled otocephalics of the higher grades in the complete absence of the lower jaw and zygomatic arches and the absence of the tooth-bearing portions of the maxilla and premaxilla. Paired nostrils and paired cerebral hemispheres were, however, present at least in some. They differed radically in the characteristic type of eye defect, bilateral microphthalmia (rare in low grade otocephalics) instead of cyclopia, and in the complete absence of inner, middle and external ears. In the otocephalics of strain 13, distorted cochleae and semicircular canals were present in the most extreme cases. In these monsters of strain 2, the tongue consisted of two wholly separate ridges, projecting from the lateral walls of the pharynx in front of the hyoid. This differs from the normal tongue found in the otocephalic series except for reduction or absence in the most extreme cases.

The occurrence of only one small cluster from a mating in a late generation of inbreeding in one strain indicates the occurrence of a mutation that tends to produce (with a probability of 0.04) an acute anterior inhibition early in development but after physiological isolation of paired optic fields and thus later than the initial time for otocephaly, in which microphthalmia of one or both of the paired eyes of the lower grades was rare (three cases, grades 1, 5, and 6). The two types of abnormality probably overlap in common sensitivity of the portion of the neural crest from

which the mesectoderm of the mandibular arch, its maxillary process and the frontonasal process are drawn. The essential difference in the actions of heredity in the two cases is thus probably a slight difference in the timing of an acute anterior inhibition.

Atavistic polydactyly

We turn now to a very different type of anomaly, one that has no appreciable deleterious effect on the individual under laboratory conditions and that seems to be merely a reversion toward a remote normal ancestral condition. This is the occurrence of a more or less well-developed little toe.

The guinea pig, like all the wild species of the family Caviidae has four digits on each front foot, three on each hind foot. The thumb, little and big toes are the ones that are missing. The occurrence of an apparently atavistic little toe is not, however, uncommon. Castle ('06), Stockard ('30), and Pictet ('32) all have found it in their colonies. Castle produced by selection a strain (D) that bred true to perfect development of this digit and associated parts of the foot such as a plantar pad near its base. Among the 23 inbred strains discussed here, the character was absent in 12, sporadic in 5, and present in moderate frequencies in 6.

Castle's four-toed strain D was crossed with several of these inbred strains (Wright, '34f) (fig. 1). In the cross with strain 2 (wholly three-toed except for two animals near its beginning) there was complete dominance of the normal (three-toed) condition in reciprocal F_1's. Passable approaches to a 3:1 ratio of normal to polydactyl in F_2 and to a 1:1 ratio in the backcross to strain D, suggested that the trait depends on a single recessive gene. This suggestion completely broke down on testing the supposed backcross segregants by a second backcross to strain D. The three-toed and four-toed individuals gave almost the same result (23 and 16% three-toed, respectively). It became necessary to postulate multiple genetic factors that, acting in conjunction with nongenetic ones, put the developing embryo above or below a threshold for pearance of a rudimentary little toe a if the former, above or below a sec threshold for perfect development. A ysis indicated four pairs of alleles on assumption of equal importance or, on assumption of maximum heterogeneity gene effects, of one leading pair resp sible for half the underlying physiologi differences between the parents plus multiplicity of factors with very sli effects, for the other half (Wright, '52

Crosses of strain D with strain 13, wh had never produced a polydactyl, gave such simulation of one factor heredity in the preceding case. Thus F_1 inclu 67% three-toed and 33% four-toed. Te in F_2 and in backcrosses to strain D vealed no significant genetic difference tween the two F_1 types and gave only 46 normal in F_2, only 14% in the backcr The results could, however, readily be terpreted on the same type of hypothe as above by supposing that the genoty of strain 13 was closer to that of strain than was that of strain 2 (differing in t equivalent of two or three equally i portant factors).

Finally strain 35 itself produced ab 31% with some development of the lit toe. There were highly significant diff ences among branches (9–69% polydact several of which separated after so ma generations of brother-sister mating as indicate the rather frequent occurrence mutations with effects on the perce age, a very sensitive indicator. With branches, however, matings of three-t by three-toe and four-toe by four-toe d not differ significantly in the percentag in their offspring. On mating strain with D, F_1 showed a predominance of po dactyls and the results in F_2 and in a bac cross to 35 were again in harmony with t hypothesis of multiple factors and the t thresholds. In this case the distribution normals, of ones with rudimentary lit toes and of ones with perfect little to indicated transgressive physiological var tion and the hypothesis that strain 35 su plied one or more factors for polydacty lacking in D.

The occurrence of normals and po dactyls of various grades of perfecti

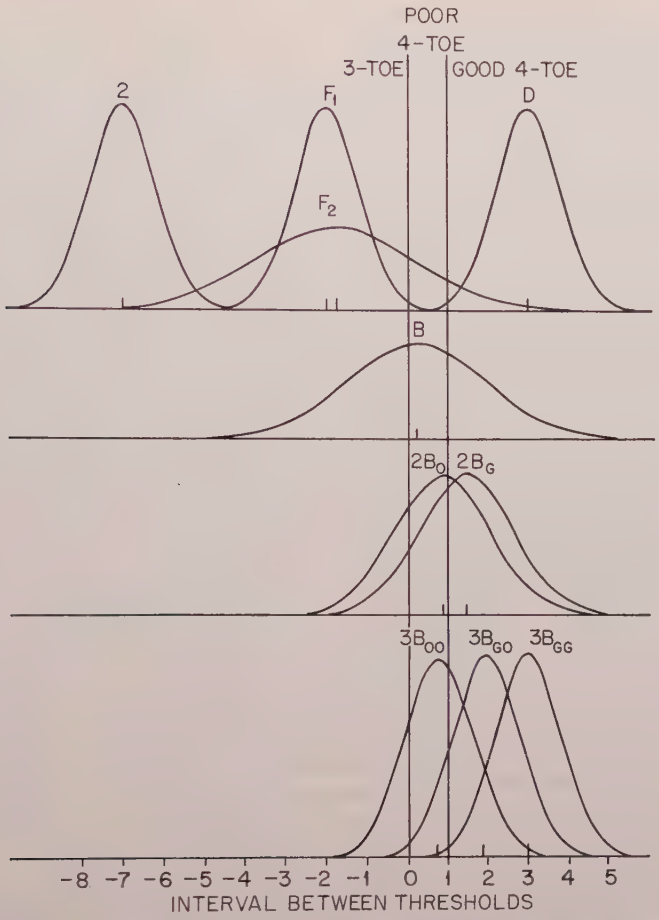

Fig. 1 Hypothetical distributions on an underlying physiological scale of inbred strains 2 and D, F_1, F_2, the backcross (B) of F_1 to D, second backcrosses to D ($2B_0$, $2B_G$) from 3-toed and good 4-toed individuals of B, and third backcrosses to D ($3B_{00}$, $3B_{G0}$, $3B_{GG}$) from 3-toed from 2,2,2 B_0, 4-toed from B_0 and 4-toed from B_G. The scale unit is the interval between the thresholds. The distributions of F_2 and all backcrosses are represented by normal curves, trichotomized by the thresholds so as to give areas equal to the observed frequencies of 3-toed, poor 4-toed and good 4-toed. The standard deviations (.80) of 2, D and F_1 are borrowed from that indicated by the trichotomy of strain 35 (not shown here). The means of D (good 4-toed) and F_1 (3-toed) are located so as to indicate the possibility of only rare occurrence of poor 4-toed in each case. The mean of 2 is located so that that of F_1 is half way between it and that of D_1.

ıong animals of the same genotype by t [same branch of strain 35, F_1 (13 ×] indicates as noted, considerable non-ıetic variability with respect to the un-ɾlying physiology. Analysis of strain indicated that the most important non-ıetic factor was the age of the mother ıgh percentage of little toes from im-ture mothers, low from mature thers). Another factor was the sea-ıal conditions. There was a relative high percentage in winter (fig. 3) (but only under the conditions at Beltsville) (Wright, '34a).

It would be possible perhaps to suppose that some or all of the genes that favor development of the little toe in strains D and 35 are ancestral ones, not wholly lost in the guinea pig or brought back by reverse mutation. We consider next, however, the effects of a mutant gene that tends to restore not only the little toe but

also the thumb and big toe for which such an interpretation seems wholly precluded. All individuals with this gene, *Px*, trace to a single individual that showed imperfect thumbs, little toes, and one imperfect big toe. Repeated backcrossing of descendants to normals of other strains demonstrated unequivocally that the mutant type (*Px/px*) differed from normals (*px/px*) by a single gene, although penetrance was incomplete (Wright, '35). About 18% of *Px/px* in the strain of origin were wholly normal, although transmitting *Px* as regularly as the polydactyls. In this strain 74% had one or both thumbs, 62% had one or both little toes, but only 2% had one or both big toes. On outcrossing to certain strains in the course of testing for linkage, penetrance fell almost to zero. On the other hand, on crossing to strain D, all *Px/px* had both thumb and little toe represented; 16% had one or both big toes. On backcrossing again to strain D, over 50% had one or both big toes. Thus the heredity of strain D not only acts cumulatively with *Px/px* with respect to the occurrence of the little toe but increases the penetrance of the thumb and big toe, digits that have never risen above the threshold within strain D by itself.

The effects of genotype *Px/px* are not wholly limited to restoring lost digits. There have been four cases with rudimentary digits that cannot be interpreted as atavistic. In three of these, there was such a digit at the base of digit V on the forefoot. In the other case, it was at the base of the thumb.

There was also some tendency toward ventral flexure of the feet (65 forefeet, 4 hind feet, 4 both). These represent between 1 and 2% of all *Px/px*, the exact percentage being uncertain because of the irregular penetrance. Those with this type of defect showed a distinctly higher mortality between birth and weaning (44%) than those without it (27%). It may be added that, although *Px/px* in general showed no significant difference from *px/px* in either mortality at birth or between birth and weaning in the strain of origin, both percentages were higher, and the percentage raised of all born significantly lower on crossing with D. In some cases, at least, there was defect of sternum in *Px/px*. These accessory defects p toward general inferiority of *Px/px*. the other hand, animals of this genot were significantly heavier (7%) t their littermates of genotype *pxpx*.

The polydactyl monster

Matings in which both parents w *Px/px* have produced 79 of an extraor ary type of monster (Wright, '34e, '3 The incidence was far below the 2 expected if this type is *Px/Px*. Sc studies ('37) of the incidence in e fetuses have, however, shown that the pected proportion is realized in these that most of this genotype die at about twenty-sixth day of gestation. In stocks that he studied, about 92% d early leaving only 8% to die at bi Thus there was apparently low penetra not because of failure of the genotype produce an effect but because of the treme degree of the effect. The mean of litter from *Px/px* by *Px/px* in the str of origin was 1.83 in comparison with 2 where one parent at least was *px/px*.

The homozygotes show a most extr dinary array of abnormalities. These h been described in detail for those t reach birth (Scott, '38) and for vari stages from the first appearance in the bryo (18½ days from conception) of o grown but underdifferentiated mid- hindbrains, and forelimb buds of dou thickness (Scott, '37). Those that re birth show the fetal C-shaped (instead S-shaped) flexure of the vertebral colu excessive subcutaneous fat, shortening all parts of the limbs except the upper a absence of tibia and rotation of the h limbs; ventral flexure of all the feet, wh are of double width and have 7–12 di each; overgrown mid- and hindbrain ing rise to hydrocephaly or exencepha microphthalmia; many defects of skull, including cleft palate, often rela externally to harelip; telescoped sternu greatly reduced digestive tract and lu and lobulated kidneys of fetal type. circulatory system showed no defects cept those directly associated with ab mal parts, and there were no gross ab malities of the reproductive system either sex. The death of most of monsters at the twenty-sixth day was

rently from hemorrhage in connective sue in the dorsal neck region back of the ergrown brain.

There seems no possibility of tracing all e abnormalities to a single localized de- ct as in the case of the otocephalic onsters. There may, however, be a ngle metabolic defect that leads more or ss simultaneously after the eighteenth y to delay in morphogenetic processes d correlated overgrowth in various pidly developing parts of the body, such brain and limb buds as well as second- y effects on other parts such as eye and eleton.

On this basis, the relatively narrow calization of the effects in the hetero- gote is to be attributed to the low thres- ld of the limb buds to the type of etabolic defect attributable to the gene. elayed morphogenesis may be interpreted leading to overgrowth and formation of excessive number of lobes. The genome guinea pigs is sufficiently like that of e remote ancestors of the Caviidae to ad to the development of as close an proach to the ancestral pentadactyl foot the number and size of the lobes per- it. If six lobes are formed on a forelimb d, a good thumb is formed opposed to e four normal fingers of the guinea pig t a rudimentary sixth finger is also rmed. If, however, the limb bud be- mes of double width under the more vere deviation from normal physiology duced by *Px/Px,* the situation is so dif- rent from that to which the genome is apted that a monstrously abnormal foot velops, unlike anything in the ancestry. If this is the interpretation to be put on e atavistic foot of *Px/px* and the mon- rous foot of *Px/Px,* it seems probable at the multiple factors of strains D and act similarly in delaying morphogenesis the hind-foot bud and permitting ough overgrowth to favor the appear- ce of four instead of three lobes. The avistic little toe and its associated antar pad then result from the action, in is situation, of the same elements of the nome essentially unchanged since the mote pentadactyl ancestors as those that t in the case of *Px/px.* The specificity all the various patterns of development the foot so far considered thus resides in a low threshold for processes that normally restrict the number of lobes on the fore and hind feet to four and three, respectively, rather than in any more specific relation of these genes to the various digits. The term "phenocopy" for little toes induced by unfavorable environmental condition or immaturity of the mother is thus somewhat misleading in seeming to imply that the genes in question have a specific relation to the character that the environment somehow copies. The more probable interpretation is that gene products and environment act in essentially the same way at the morphological level.

Microphthalmia

Among the 76,000 guinea pigs recorded at the Whitman Laboratory, there have been 145 cases (0.19%) of microphthalmia and anophthalmia after excluding the 79 cases of *Px/Px* and including only those with eyes definitely small or absent. The many cases of opaque lenses, some of which later cleared up, are excluded. The 145 cases include 16 in which microphthalmia was associated with other defects (7 of leg or foot, 3 of jaw, 1 of both leg and jaw, 3 hydrocephalics, and 2 otocephalics). The frequency of associated defects (12%) is far above the incidence of these defects by themselves (0.2%) and thus indicates a common developmental factor. It may be added that nonmicrophthalmics from the same matings as microphthalmics (1673 in number) also included an excessive number (16, or 1.0%) of other defects (2 with the chunky-flexed foot syndrome discussed later, 5 others with flexed feet, 2 otocephalics, 2 exencephalics, and 1 hydrocephalic, 2 with umbilical hernia, 1 with jaw defect, and 1 with abnormal female genitalia). These also indicate that elements of heredity that contribute to the occurrence of microphthalmia may also contribute to very different abnormalities. The sex ratio among the microphthalmics like that in otocephalics, shows a marked excess of affected females (52♂:90♀:3 undetermined, including 6♂:10♀ in the compound cases). These are the only abnormalities in which significant deviations from equal numbers of the sexes was

found. Five of the 16 compound abnormalities were born dead, 8 died before a month of age, leaving only 3 that were weaned. Even the uncompounded microphthalmics showed a somewhat low percentage reared of those born alive though the significance of the difference is doubtful (table 1).

The 145 microphthalmics were born in 125 litters that produced 419 young. Thus there were 294 littermates of microphthalmic propositi, one from each litter, of which 20 or 6.8% were microphthalmic. This proportion (p′) is about half that (p = 13%) in a population of which these litters are a random sample. $kp/[1-(1-p)^k] = 1+(k-1)p'$, $p \approx 2p'$ for small kp, where k is size of litter. The microphthalmics came from 110 matings that produced 1818 young. Taking one microphthalmic propositus from each mating there were 18 cases of microphthalmia among 1470 sibs from other litters than the propositus or a probability of 0.012 of microphthalmia, six times the general incidence and highly significant. This figure probably indicates a small genetic component whereas the higher probability for littermates indicates a considerable environmental component. A genetic component was also indicated by difference in incidence among inbred strains in the Beltsville data, 1906–15, 1.6% (= 8/504) in strain 38, 0.1% (= 8/8399) on the average in seven other strains, none (0/11,135) in 14 other strains as well as 0.2% (= 4/2071) in the control strain. The difference between strain 38 and the others is highly significant. Eaton ('37) has described a cluster that appeared in the color experiments after 1925 in which a high incidence was clearly hereditary.

There is, of course, no indication of the mode of inheritance and no assurance that it was always the same heredity that was involved. It was certainly not the same heredity when microphthalmia occurred i *Px/Px* or in the anotic monsters. We a probably again dealing with heterogeneo arrays of genetic and environmental fa tors that happen to produce sufficient i hibition or other disturbance, during t critical period for development of the ey to overcome developmental homeostas The only common element is this point weakness in the developmental process.

The chunky type of monster

There have been 26 records at the Wh man Laboratory (frequency 0.03%) of type of monster with a chunky body, som two-thirds of the normal length but n differing significantly in weight from no mal littermates (average difference: 1 g lighter in paired comparisons of eig litters, $t = 0.4$). This has been associate with conspicuous abnormality of some all of the legs, with three doubtful exce tions. The feet, especially the forefee tend to show ventral flexure and the hin legs sometimes appear to be twisted. Th sex ratio is normal (11 ♂:13 ♀:2 undete mined).

Twelve of them came from seven closel related matings (66 young) that traced t a cross between the inbred polydact strain D and a self-contained strain pink-eyed dilute brown tricolors (B neither of which had produced monsters this type and very few abnormalities of an type, except for the little toe of strain D There were, however, four cases of le abnormality, somewhat like that of th chunky monsters, and one case of microph thalmia in strain B. There were 7 young in F_1 of the DB cross, all normal and 415 in F_2 including eight chunkies an seven other abnormalities, all but one o the latter from a single mating discusse later, and 44 young from matings of type $F_1 \times F_2$ or F_3 including four chunkies There was clearly a genetic basis eve

TABLE 1

	No. of animals	Percentage		
		Born alive	Raised of live-born	Raised of total born
Normal	1673	81.7	86.7	70.8
Microphthalmia only	129	79.8	78.6	62.8
Microphthalmia plus other defect	16	56.3	33.3	18.8

ithin this group, but the monsters cannot
e interpreted as attributable to a simple
cessive of full penetrance.

One of the matings (DB 59) produced
ie most extraordinary array of diverse
onsters observed from any mating in the
story of the colony. Its 32 offspring in-
uded 2 chunkies with abnormal legs, 3
ith leg defects only (1 with palmar flex-
e only, 1 with contorted hind legs, and
with both defects), 1 with palmar flex-
e, missing digits, and anophthalmia, one
ith anophthalmia only, and 1 grade-2
ocephalus. These 8 monsters were scat-
red through 6 of the 10 litters. It may be
oted that 6 of the 8 had leg defects (of
rious sorts) as a more or less common
ature and only the otocephalus has a
fect that is not associated in some case
ith leg defect. This clustering seems too
uch to be attributable wholly to coinci-
nce. It may be suggested that a genetic
mplex, possibly including elements of
ie system that restores the little toe in D,
nd a gene derived from B happened to be
ssembled in the animals mated in DB 59
at was of such a nature as to lead to
sturbances in development that might
fect different parts (fore and hind legs,
cial skeleton, eyes and perhaps pre-
notdal mesoderm, depending on the exact
enotype of the individual and environ-
ental conditions. It should be said, how-
er, that the only abnormalities recorded
om other matings of the group were the
) others with the chunky-leg defect syn-
rome, 1 with leg defect only, and 1 with
icrophthalmia. There was also no signifi-
nt association of abnormality with pres-
nce of the little toe.

The remaining 14 with the chunky-leg
fect syndrome were scattered among 12
atings (132 young) with no significant
lations to each other or to the DB cluster.
part from two cases of leg defect alone
ere were no other abnormal individuals
om these matings. One of the monsters
this group had severe facial defect (no
axilla, short mandible) in addition to the
unky body and short clubbed legs. The
currence of two litters in this group and
ree in the DB group with two chunkies
ch (with only two normal littermates al-
gether) suggest an environmental con-
bution to the abnormal development.

Flexure of the feet

In the Whitman data, there have been 50 cases of ventral flexure of the feet (29 anterior, 8 posterior, 13 both) sometimes associated with torsion of the legs, after excluding the 79 polydactyl monsters (*Px/Px*), the 73 cases in which this character was probably an occasional byproduct of *Px/px*, the 23 chunky monsters and five nonchunkies with this character from matings that produced chunkies. Two of these 50 also were hydrocephalic, and one had both protruding brain and defective digits. Three were also microphthalmic compounded in two cases with gross facial defect.

These 50 cases came from 41 different matings among which there was little indication of significant relationship. There was, moreover, only one case in which the defect appeared in two different litters (one individual each). Taking one propositus from each mating, these had 355 sibs that were not littermates, indicating a probability of only 0.003 that an individual in another litter from the propositus would be similarly defective. There were 92 individuals in litters that contained this defect. Taking one propositus from each of the 42 litters, the frequency in littermates was 8 in 50 or a probability of 0.32 of the defect in a littermate of an affected individual.

It appears that the occurrence of this defect is caused, to an overwhelming extent, by intrauterine environment in cases in which it is not an effect of *Px* or a manifestation of the chunky syndorme. It should be noted that the four nonchunkies with leg defect from mating DB 59 appeared in three different litters and may well have depended on incomplete penetrance of the heredity for the chunky syndrome.

The mortality records of the abnormals, their normal littermates, and normal sibs from other litters are shown in table 2.

The abnormals are clearly inferior to their normal sibs and there is an indication of lower vitality after birth of the normals from the same litters as the abnormals than of the normals from other litters.

TABLE 2

	No. of animals	Percentage		
		Born alive	Raised of live-born	Raised of total born
Normals (other litters)	353	74.8	79.2	59.2
Normals (same litter)	42	81.0	61.8	50.0
Flexed feet	50	66.0	45.5	30.0

Abnormal digits

There have been 21 records of abnormalities of the digits at the Whitman Laboratory, excluding those with atavistic polydactyly. These include cases of distorted digits, partly fused digits, missing digits, and two cases of symmetrically doubled digits, a wholly different type of polydactyl from the atavistic sort. There was one case of a chunky with palmar flexure, two other cases with palmar flexure, one case of an undersized foreleg with no digits, and one association with microphthalmia. The sex ratio was normal (10 ♂:11 ♀).

Since no mating produced more than one and there were no significant relationships among them, there is no direct evidence that heredity played a role. There is, however, evidence of association with other defects beyond that expected by chance.

Micromelia and dropsy

A type of still born monster was recorded in the Beltsville data (Wright, '22a) in which the body was undersized and the legs rudimentary. In extreme cases, a leg was represented externally merely by a single claw. Five were produced in inbred strain number 24 and one each in two other strains. Two of those in strain 24 were born in different litters from a mating in the eighth generation (18 young). Three matings were made, of which 2 produced only normals (6, 24) but the other produced 3 of these monsters in different litters in a total of 13 young. All micromelics had one or more normal littermates. This history strongly suggests segregation of a simple Mendelian recessive (26 normal:5 micromelic) that had arisen as a mutation in the course of the inbreeding.

Four cases of micromelia (also all born dead) were recorded from the Whitman Laboratory. In one the head was abnormally broad, in another only the forele were diminutive (only toes visible ext nally), and in the two other cases micr melia (only toes visible on all four limb was associated with a grossly dropsic condition of the tissues, presumably ind cative of kidney defect. There was rath close relationship in only the last tv cases. One of these had 2 normal si (same litter) the other had 33 norm sibs, of which 6 were in the same litter the monster. Heredity may have played role in this case but not segregation of single gene of full penetrance.

In addition to the two cases of drop associated with micromelia, there ha been three of dropsy alone (all born dead from a single brother-sister mating (1 young). Two were in the same litter wit out normal littermates but the other wit a birth weight of 221 g had a normal li termate weighing 84 g. Three lines of i breeding from this mating and its pare mating failed to yield any more monste of this type. The segregation of a Mende ian recessive is suggested in this last ma ing, though less forcibly than in the micr melia of strain 24.

Exencephaly and hydrocephaly

There have been five records of protru sion of the brain (two born alive, thre born dead) from three unrelated mating at the Whitman Laboratory (excludin *Px/Px*). One mating (6 offspring) pr duced 2 exencephalics in a litter of 3. An other (with 30 offspring) produced 1 ex encephalus in a litter of 4 and both a exencephalus and a hydrocephalus in an other litter of 4. The occurrence in tw litters (fourth and eighth) suggest heredit and the occurrence of exencephaly and th probably related type of defect hydr cephaly in one litter and of two exenceph alics in one litter of the other matin suggest effects of the uterine environment

There have been 20 cases of hydrocephalus in the Whitman data, including the one just referred to. These have come from matings (236 offspring) with probably significant relationship in some cases. One mating produced 3 (among 30 offspring), in a litter of 2 and 2 (both with palmar exure) in a litter of 4. Another with 15 offspring produced 2 in a litter of 4. Three were both hydrocephalic and microphthalmic. Another had two microphthalmic ones. Again we have indication of contribution from both heredity and uterine environment and of association with other defects.

Facial defects

Four rather closely related matings (88 young) produced one each of a type with harelip and abnormal or missing upper incisors and no other abnormalities. Four other miscellaneous facial defects came from four unrelated matings.

Miscellaneous defects

There were four unrelated cases in which one or both external ears were conspicuously small.

There were four cases with viscera protruding from an umbilical hernia and one case in which the heart was exposed because of imperfect development of the chest wall. One case of spina bifida was recorded.

Anemia and sterility of "silver-whites"

Genotype *sisiDmDm* (silver) exhibits a more or less extensive sprinkling of white hair in the coat, and some dilution of the colored hairs, neither of which is progressive. The amount of silvering varies from a few white hairs on the belly to white sprinkled with colored hairs on head and legs, according to easily selected modifiers. Traces of silvering may occur in heterozygotes. The character is presumably caused by death of pigment cells at a critical period of development, but there are no appreciable unfavorable effects on the individual as a whole.

The genotype *SiSidmdm* (diminished) has no recognizable effect in the presence of the normal allele C of the albino series but is responsible for accentuating the dilution of color in lower compounds at the c locus. No injurious effects on the individual have been noted.

The combination, *sisidmdm*, is self white except for occasional patches of very pale color on the head. Eye color is slightly reduced. This combination comes up for consideration here because these animals are anemic (75% of normal hemoglobin content), have a significantly higher mortality rate after birth than their normal littermates, and especially because of sterility. The males have been completely sterile as far as observed (17 tested, none of 18 normal littermates sterile) with testes about 25% of the normal weight and no spermatogenesis. The females have been sterile in about 50% of the observed cases (10 out of 21) and those that were fertile were less productive than their normal littermates (of which none of the 26 tested was sterile). This unfavorable deviant is interesting as dependent on two loci (Wright, '60).

II. QUANTITATIVE VARIABILITY IN VITAL CHARACTERS

We come now to the genetics of quantitative differences in vital characters. We will consider two aspects of fecundity—the number of litters per mating year and size of litter; two aspects of viability—the percentage born alive and the percentage raised to 33 days of those born alive; and two aspects of early growth—birth weight and rate of gain to 33 days of age. The total mortality curves (Eaton, '32) and the growth curves of controls, inbred strains and crosses (McPhee and Eaton, '31) have been studied but will only be touched on here.

The most important factor affecting perinatal mortality and early growth is size of litter. Analysis by strains has been given by Wright ('22a, b). Figures 2 and 3 show the averages for the entire inbreeding experiment from 1906 to 1924 as reported by Haines ('31). It may be noted that there are strongly negative but curvilinear relations of the weights at birth and 33 days to size of litter. The viability percentages show optima in litters of two.

Under favorable conditions the optimum size of litter was three with four only slightly inferior. Table 3 brings this out

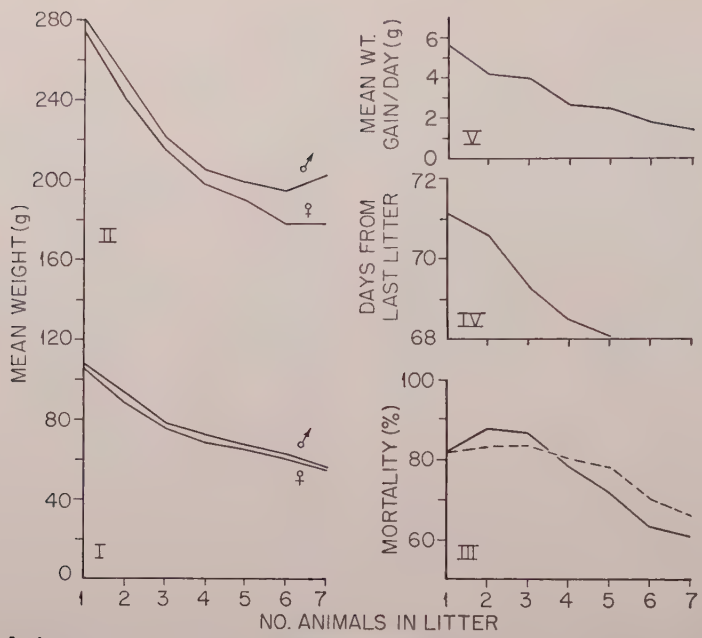

Fig. 2 Relations to size of litter of weight at birth (I) and at 33 days (II) (Haines), mortality percentages (III: —, born alive; – – –, raised of live-born) (Haines), mean interval from last litter, 65–75 days (IV) (Eaton), and fetal gain per day from fifty-fifth to sixty-fifth days (V) (calculated from Ibsen).

for percentage born alive of the total inbreds of three periods and of the controls for the two of these periods in which they were recorded. Conditions were much less favorable in 1916–18 than before.

A second important factor is season of birth. Haines found the averages for the various vital characters corrected for size of litter, for all inbreds born in each of the twelve months for the period 1906–24. These averages (by month of birth) are shown in figure 3 together with the average percentage of animals with the normal three toes on the hind feet in strain 35 discussed in part I.

Litters per 100 matings for the various months seem to have been regularly thrown into oscillations after high frequencies born in June, July, and August (conceived 68 days earlier). The other characters are in general higher in summer than winter, but the peak effects are somewhat out of step. The peaks for postnatal gain and percentage raised of those born alive are naturally found in animals born somewhat earlier in the year than in the case of birth weights and percentage born alive, which in turn have earlier peaks (by month of birth) than frequency or size of litter.

The differences between conditions in different years were almost as important as those between seasons of the same year. Conditions seem to have been rather favorable from 1906 to 1915 but were decidedly unfavorable during 1916 to 1918. They improved irregularly from 1919 to 1924. Figure 4 shows the record of the control stock and the four most numerous inbred strains with respect to 33-day weight and size of litter for the years 1916 to 1924 after 9 years of brother-sister mating and elimination of all early branches (Wright and Eaton, '29).

Since it is unlikely that there had been any appreciable genetic change in the control stock since 1906, the marked changes in the averages are assumed to reflect changes in the environmental conditions. This is confirmed by the largely parallel changes in the inbred strains. The averages for the other characters behaved similarly though no other showed as close parallelism as did 33-day weight. It may be noted that the conditions favoring one

TABLE 3

Percentage born alive by size of litter in the inbreds during three periods and in the control stock in two periods

	1		2		3		4		5		6	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Inbred												
1906–10	407	87.0	864	89.3	954	89.4	546	84.3	244	80.5	61	70.2
1911–15	780	81.6	1366	88.5	1289	87.3	565	80.2	143	65.3	39	57.7
1916–18	331	81.6	622	81.7	444	73.8	129	57.6	22	39.1	2	50.0
Control												
1911–15	69	82.6	170	87.6	237	90.8	122	89.8	56	86.8	23	74.6
1916–18	70	82.8	147	90.5	166	84.7	62	79.4	10	78.0	2	16.7

character are not necessarily the same as those favoring another. Thus 1918 and 1922 were poorer years for size of litter than for 33-day weight (corrected for the effect of size of litter).

The systematic differences among the inbred strains indicate that genetic differentiations had occurred among them. Paired comparisons for these 9 years demonstrated the existence of significant differences in all the vital characters studied and also significant inferiority of the inbreds as a group to the controls.

A fourth factor of importance was the condition of the dam. There was a correlation between weight of dam and size of litter of $+ 0.419 \pm 0.052$ and ones between weight of dam and birth weight of 0.428, 0.512, 0.619, and 0.740 (average 0.575) in litters of 1, 2, 3, and 4, respectively (Eaton, '32).

At this point it will be well to consider how far the marked genetic differences among the strains in the various characters (corrected for effects of size of litter) are independent and how far merely indicators of one general character, vigor (Wright, '22a). It is not surprising that the averages for birth weight and gain were strongly correlated ($+ 0.75$) in the 23 strains that were on hand in the period 1906–10, and this was also true ($+ 0.59$) in the period 1911–15 when 22 were still on hand. The order among the families established at birth persisted in the main throughout later growth. There is clearly much common heredity for growth at all ages. The percentage born alive and the percentage raised of those born alive on the other hand, showed little or no correlation with each other in either of the above periods ($+ 0.03$, $+ 0.30$) and this was also true of the two components of fecundity ($+ 0.04$, $- 0.03$). Among the other correlations between vital averages among these strains, only two showed significance in either period, size of litter with corrected birth weight ($+ 0.26$, $+ 0.62$) and size of litter with corrected gain ($+ 0.37$, $+ 0.62$). There is clearly some common heredity for size of litter and growth rate (for a given size of litter), a relation that has also been found in rabbits (Gregory, '32) and in mice (MacArthur, '49). The composite character,

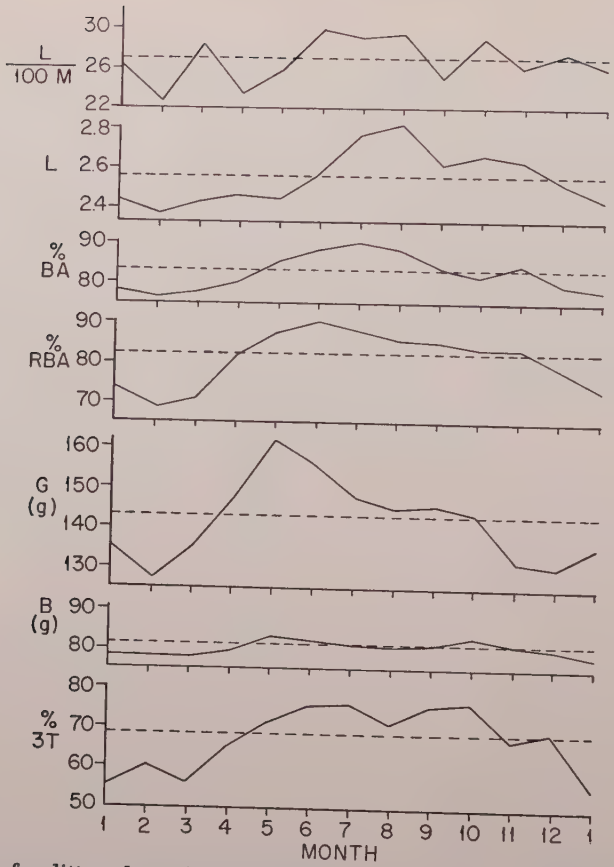

Fig. 3 Means for litters born in each month of the year of litters per 100 matings (L/100 M), size of litter (L), percentage born alive (BA), percentage raised of those born alive (RBA), gain to 33 days (G), birth weight (B) in total of inbred strains (Haines). The last four are corrected for effects of size of litter. The bottom graph (3T) shows the percentage of young with three, instead of four, toes on the hind feet in inbred strain No. 35.

percentage raised of all born, showed correlations of − 0.04 and − 0.31 with 33-day weight (both for given size of litter) and of + 0.03 and + 0.29 with the composite fecundity, young per mating year. The latter showed correlations of only + 0.21 and + 0.22 with corrected 33-day weight in spite of the significant correlations of its component, size of litter, with both growth characters. To a large extent, the deviations of the various vital characters are inherited independently after allowing for the direct effects of size of litter.

There was a considerable tendency for the ranks of the 22 strains in the period 1911–15 to agree with their ranks in the earlier period (average correlation + 0.50). The most important exceptions are found in strains 35 and 2, both of which ros from low to high rank in several respects Inspection of the pedigrees showed tha this could be interpreted in both cases a caused by displacement of branches wit low averages by expansion of a single earl line with high averages. By 1917, strai 35 traced entirely to a single mating in th twelfth generation of brother-sister matin and the other strains were not far behind The records for the period 1916–24 indi cate that the decline of the inbreds, rela tive to the control stock, had practicall come to an end by the beginning of thi period and that each had become almos fixed in its genetic constitution (Wright an Eaton, '29). Nevertheless, there were considerable number of significant trend

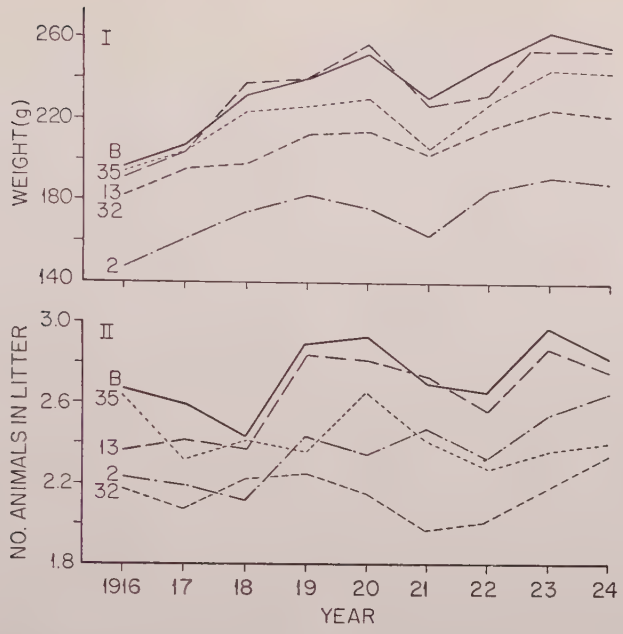

Fig. 4 The means for weight at 33 days (I) (corrected for size of litter) and of size of litter (II) in each year from 1916 to 1924 for the control stock B and the four most numerous inbred strains.

particular traits, relative to the control ock. This is illustrated in figure 4 with spect to size of litter by the rising trends strains 2 and 13 and the falling trend strain 35. The latter was definitely the ost vigorous of all the strains in 1916 in ost respects; strain 2, although relatively gh in rank among all strains at that me, was lowest in weight and next to west in size of litter among the five that ere maintained after initiation of an ex-nsive program of crossbreeding. In the urse of the period 1916–24, strain 35 st rank in most respects and strain 2 se, with significant differences in trend both viability percentages, in size of ter and especially in the composite char-ter, young raised per mating year. It ay be added that strain 2 exhibited a ore favorable mortality curve throughout e than any other inbred strain (Eaton, 2) and became the easiest strain to aintain. Inspection of the pedigrees in-cated that these late changes could not accounted for by displacement among anches or by mutation. It seemed neces-ry to conclude that they depended on specific responses of the various heredities to changing conditions.

Fixation of heredity in an inbred line manifests itself in different ways for different characters. There may be complete phenotypic uniformity as in the case of quality and intensity of coat color and, to almost as great an extent, in conformation. In other cases, illustrated in these inbred strains by amount of white in the spotting pattern, there may be enormous variability within each but no correlation between parent and offspring and constancy of the mean. In this case variability depends largely on developmental accidents rather than on tangible environmental factors. In the case of 33-day weight, relative rank becomes firmly established as illustrated in figure 4 but, in addition to much individual variability, the mean varies greatly from season to season and from year to year. Finally we have the situation in the vital characters other than weight in which the means shift in rank from time to time, probably not because of genetic changes but because of nonadditive genotype-environment interaction.

Further light on the genetic basis of these vital characters was provided by crosses among the inbred strains (C0) followed by renewed brother-sister mating for two generations (C1, C2), by mating of crossbred males to females of a third strain (CA), the reciprocal cross (AC), and second generation crosses that brought together four inbred strains (CC) (Wright, '22b).

In order to make valid comparisons, we obtained the averages for all inbreds (largely five strains) for each 3-month period in the years 1916–19. The number of young produced in each crossbreeding experiment in each of these periods was found and used as the basis for properly weighted averages of the total inbreds produced simultaneously. Some of the results are shown in figures 5 and 6. These figures show the percentage deviations of the five inbred strains and the miscellaneous inbreds (M), the control stock (B) and the above crossbreeding experiments in each case from the aver of its contemporary inbreds.

It may be seen that birth weight (given litter size) depends largely (ab three-fourths) on whether the moth was inbred (A, C0, CA) or crossbred (A CC, C1), the latter groups having cidedly higher averages. Decline beg with renewed inbreeding of the *moth* (C2). Gain from birth to 33 days, the other hand, depends to a much lar extent on whether the individual its is inbred or crossbred, the latter havi the higher averages. The weights at year of age show smaller differenc among the inbred strains than the ea gains but still show marked heterosis the first cross (C0) half of which is l on renewed inbreeding (C1). A speci difference between strains 2 and 32 relative weights of males and females of interest.

Figure 6 shows similar compariso for frequency and size of litter, the t

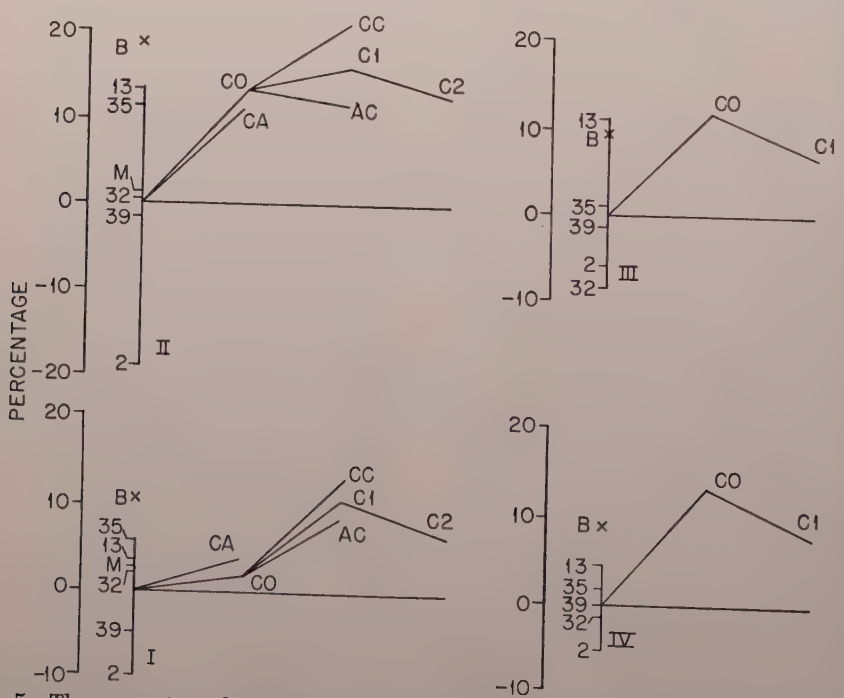

Fig. 5 The percentage differences from contemporary inbred average in weight at birth (I: all born) and gain to 33 days (II) among five inbred strains, miscellaneous inbreds (M), controls (B) and crosses: C0 (first crosses), CA (C0 ♂ × unrelated inbred ♀), AC (inbred ♂ × unrelated C0 ♀), CC (C0 ♂ × unrelated C0 ♀), C1 (CO × CO littermates), C2 (C1 × C1 littermates). The weights of males (III) and females (IV) at one year are also shown.

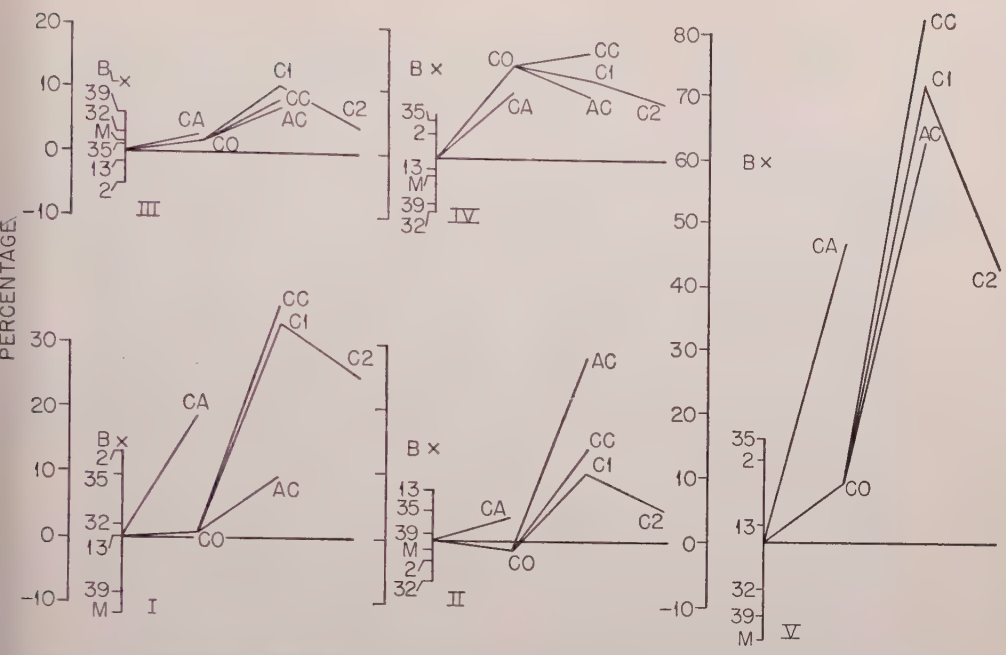

Fig. 6 Percentage differences from contemporary inbreds in litters per year (I), size of litter (II), percentage born alive (III), percentage raised of those born alive (IV)—both percentages corrected for size of litter, and the composite variable, young raised per year (V). Symbols as in figure 5.

ıbility indices (corrected for effects of e of litter) and the resultant of all ese, the number of young raised per ıting year. Size of litter and percentage rn alive are, as might be expected, gely maternal characters showing ap- eciable heterosis only if the mother is ssbred. Regularity in producing litters improved by crossbreeding of either the e (CA) or the dam (AC) but the sire s about twice as important in these ta as the dam. There is summation of effects of heterosis of both parents if h are crossbred (CC, C1) with decline both come from one generation of ther-sister mating (C2).

Returning to size of litter, one striking eraction effect is indicated that was apparent in the correlations among red strains. Crossbred females that duce litters relatively infrequently be- ıse mated with inbred males (AC) duce much larger litters than similar nales that are producing litters more ularly because mated with crossbred les (CC, C1). In about half the cases, ception of a new litter occurs on the day a litter is born. Delay favors production of a larger litter. There may be some reciprocal action.

The four-strain crossbreds (CC) are in all cases superior to the best of the inbreds and either about equal or definitely superior (in frequency of litters) to the control stock. At first sight this suggests overdominance, but comparison with the results for the compound character, number of young raised per mating year (fig. 6) indicates a different interpretation. In the latter character, shown on the same scale in figure 6 as its components, the superiority of CC to the inbreds is built up to more than 80% in contrast with about 36% in litters per year and less in the other components. The differences among the inbreds are much less. The reason is obvious. The ranking of the inbred strain in the four components shows little correlation but the crossbreds are superior in all respects. "The four component characters are themselves, doubtless, highly complex genetically. If further analysis were possible it might well turn out that dominance of the fac-

TABLE 4

Correlation between different litters of the same mating in the control stock B with respect to size of litter, mean birth weight and mean gain to 33 days

	Consecutive litters		Nonconsecutive litters	
	No.	r	No.	r
Size of litter	833	−0.011	2313	+0.068
Mean birth weight	833	−0.052	2313	+0.060
Mean gain to 33 days	601	+0.224	1663	+0.063

tors tending toward vigor in each respect is not even perfect" (Wright, '22b). The control stock, B, was also equal or superior to the best of the inbred strains in each case. This builds up to a 60% superiority in the compound character over the average of the inbreds.

The alleles responsible for the differences among the inbred strains were presumably segregating within the control stock from which they were derived. This, however, does not lead to much correlation between litter sizes or mean litter weights of different litters from the same mating in this stock (table 4). This is because environment plays such a gre role in the characteristics of a sing litter.

It is instructive to try to represent t principal interactions among the chara teristics of litters in a path diagram a to determine the path coefficients (Wrigl '21, '34c) (fig. 7).

Minot (1891), on finding that the bir weight of guinea pigs tended to vary i versely with size of litter, pointed out th this might either be an effect of prena competition or of a stimulus to early pa turition by a large number in a litter. I found that the gestation period did

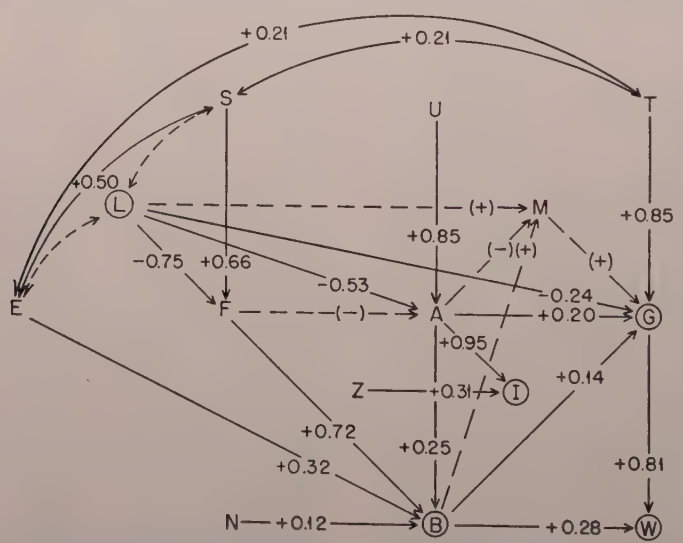

Fig. 7 Path diagram showing factors back of weight at 33 days (W) as the sum of birth weight (B) and gain to 33 days (G). B is represented as determined by early embryonic growth (E), fetal growth (F), conception age at birth (A) and nonlinear deviations (N). The observed interval since the preceding litter, if less than 76 days, (I) is represented as determined by A and errors of observation (Z). F is represented as determined by size of litter (L) and residual factors (S), A is represented as determined by L, F (negligible) and residual factors U. Gain (G) is represented as determined by the net direct and indirect effects of L, A and B and residual factors T. The indirect effects are those through perinatal mortality (M). Correlations are indicated among the ultimate growth factors E, S and T and between the first two and L. The coefficients are the estimates for the control stock B 1910–15 on using the paths indicated by solid lines.

ct tend to vary inversely with size of ter and that birth weight tended to vary rectly with gestation period. He con- uded from these and other considera- ons that deviations in the gestation riod, induced by number of fetuses, ther than prenatal competition was the planation of the inverse relation be- veen birth weight and size of litter.

Data bearing on this question were otained in 1916 from the records of the ntrol stock 1911–15 and separately om eleven of the inbred strains 1906–15. milar data were obtained later for the ntrol stock in the period 1916–18 in hich conditions were much less favor- ble. The means, standard deviations and rrelations are given in table 5 in which ie results for the inbred strains are veraged.

The correlations bear out Minot's obser- ations. We can, however, give only a ther rough path analysis since the data re not wholly suitable for various rea- ons. The number of litters in which oung reached weaning age, permitting etermination of average gain in each tter, was naturally smaller than that for hich average birth weights in litters ere available. The gestation period is presented approximately by the interval nce the preceding litter if less than 6 days, since estrus follows immediately fter parturition and does not recur for bout 17 days. The number of cases in hich this interval can be used is much naller than the number in which the her characters are available. From the andpoint of consistent path analysis, en- ies should have been restricted to those tters in which all four variables were vailable. This, however, involves a selec- on that might distort some of the rela- ons. Comparison of the various means nd standard deviations for each character ithin a set does not, however, indicate ny consistent pattern of selection in the ata as taken. A rough analysis seems arranted.

A portion of these data (inbred strains[3]) as used in an earlier paper (Wright, '21) illustrate the use of path coefficients in

[3] The figures used in 1921 have been modified ghtly by use of Sheppards correction.

TABLE 5

The means, standard deviations, and correlation coefficients in correlation arrays of litter size, interval since preceding litter (if less than 76 days), birth weight and gain to 33 days in the control stock (B) in 1910–15, 1916–18, and in the average of 11 inbred strains (1906–15)

	B 1910–15				B 1916–18				Av. of 11 inbred strains 1906–15			
	No.	m	σ	r	No.	m	σ	r	No.	m	σ	r
Interval (days)	261	69.3	1.88	−0.500	167	69.1	2.07	−0.476	904	68.8	1.87	−0.450
Litter		3.17	1.50			2.49	1.04			2.91	1.26	
Birth weight (g)	587	83.3	18.7	−0.673	459	76.9	20.8	−0.656	2307	83.3	19.1	−0.665
Litter		3.00	1.26			2.58	1.04			2.74	1.14	
Birth weight (g)	261	82.2	19.5	+0.507	167	78.3	18.4	+0.485	904	83.0	18.0	+0.569
Interval		69.3	1.88			69.1	2.07			68.8	1.87	
Gain (g)	513	152.5	49.6	−0.438	373	137.1	43.1	−0.520	2123	157.5	45.0	−0.347
Litter		3.07	1.20			2.57	0.97			2.77	1.11	
Gain (g)	224	147.3	48.3	+0.380	145	136.8	41.3	+0.306	844	156.0	43.8	+0.262
Interval		69.2	1.88			69.0	2.10			68.7	1.74	
Gain (g)	513	152.5	49.6	+0.550	373	137.1	42.1	+0.607	2123	157.5	45.0	+0.531
Birth weight (g)		84.3	17.3			80.8	18.8			84.3	17.8	

evaluating Minot's two hypotheses by representing both in the same path diagram. Birth weight was represented as completely determined by the prenatal growth curve and the time at which this is interrupted by birth. The path coefficients came out + 0.863 and + 0.327, respectively. Those relating prenatal growth and gestation period to size of litter came out − 0.594 and − 0.444, respectively. Thus the compound coefficient relating weight to litter size by way of rate of prenatal growth was − 0.513 (= − 0.594 + 0.863); that by way of shortening of the duration of growth was only − 0.145 (= − 0.444 × 0.327) and thus much less important, contrary to Minot's conclusion.

The diagram was, however, rather seriously oversimplified in order to get a solution at all. Prenatal growth might be expected to have a direct (negative) effect on time of birth, as shown in a preliminary path diagram in the 1921 paper. The relation of fetal growth to size of litter is, moreover, more complicated than was implied, a point brought out clearly in a study made later by Ibsen ('28) of weights of fetuses at successive conception ages. Ibsen found that there was no relation of weight to size of litter up to the fiftieth day (mean weight: 36.3 g) and not much even at the fifty-fifth day (mean weight: 49.4 g). After this there was rapid divergence in negative relation to size of litter (fig. 2–V).

On calculating from his data, the regressions for each size of litter from age 55, weight 49.4 g, as origin, and smoothing by least squares, the estimated daily rate of fetal gain (F) near the end of gestation comes out 5.739 − 0.664 L in grams where L is size of litter. The mean weight at conception age A, which may be taken as the end of the gestation period, thus giving the mean birth weight ($\overline{B}$), could then be estimated as approximately

$$\overline{B} = 49.4 + (5.739 - 0.664\ L)\ (A - 55).$$

Since the observed interval between litters in cases in which conception immediately follows birth of the preceding litter is about a day longer than the true gestation period, taking account of errors recording at both ends we may write

$$B = E + F\ (I - 56),$$

in which E is the variable fetal weig at the fifty-fifth day, F is daily rate gain as a linear function of litter si and I is the observed interval betwe litters. This, of course, represents wh is really a set of curvilinear growth curv after age 50 by a single line for all litt sizes from ages 50 to 55, followed diverging lines for the different litter size but should do as an approximation.

The formula involves a nonlinear ter the product of variables F and I, whi requires introduction of still another fa tor, N (= δF δI) into the diagram ba of birth weight

$$\delta B = \delta E + \overline{F}\delta I + (\overline{I} - 56)\ \delta F + \delta F\ \delta I.$$

The correlation between the observ mean birth weight of litters of each si and the best linear estimate of this fro size of litter, in the control stock 1910– was 0.985. This indicates 3% (= 1 − r determination of birth weight by devi tions from linearity. Applying 0.173 ($\sqrt{1 - r^2}$) to the observed values of r_{BL} the three sets of data gives values of p of 0.116, 0.113, and 0.115 respectivel The value 0.115 will be used throughou for this rather unimportant path coef cient.

Since there is no possibility of estimati the influence of variability in fetal weig before the effect of litter size on birt weight begins (p_{BE}), it will be well to mak a rough estimate from Ibsen's direct o servations of fetal growth. For this pu pose we will take the ratio of the standar deviation of mean weight in litters at ag 50 (rather than age 55, at which sligh effects of litter size were already apparent to that at 65 days (3 days before averag age at parturition). The variance of mea weights in litters was obtained from a analysis of variance of Ibsen's data (tab 6).

As already noted, the gestation perio (A) was not observed directly, and the ob served interval between litters is in erro by fractions of a day at each end. It ma be estimated that the correlation betwee I and A is about 0.95. All observed corr

TABLE 6

Age	No. of litters	Variance of mean weights
days		*g*
50	6	13.4
55	8	39.6
60	13	63.7
65	37	129.1

We obtain the estimate $p^2_{BE} = 13.4/129.1 = .104$, $p_{BE} = 0.322$.

tions involving interval should thus be vided by 0.95 to estimate those involving . The correlations in table 5 are the ncorrected ones involving I.

At this point, we will make an estimate n the same basis as in the 1921 paper xcept for introduction of the coefficients $_{IN} = 0.115$, $p_{BE} = 0.322$. Thus assuming $_F = 0$, $r_{ES} = r_{EL} = r_{LS} = 0$, there are six her paths in the portion leading to B. hese can readily be solved from the six quations provided by each set. These are s follows, representing the coefficients as table 7.

$$r_{IL} = 0.95\ a_1$$
$$r_{BL} = b_1f_1 + b_2a_1$$
$$r_{BI} = 0.95\ (b_1f_1a_1 + b_2)$$
$$r_{FF} = 1 = f_1^2 + f_2^2$$
$$r_{AA} = 1 = a_1^2 + a_3^2$$
$$r_{BB} = 1 = b_1^2 + b_2^2 + 2b_1b_2f_1a_1 + (0.115)^2 + (0.322)^2$$

Solution yields the estimates shown in he first columns in each set in table 7. he results in these three sets do not differ y amounts that can be considered im- ortant. The values of the compound ath coefficients relating birth weight (B) litter size (L) by the two routes con- dered by Minot are as shown in table 8. The conclusions from the earlier analysis re essentially unaltered by introducing ariables E (fetal weight at 55 days) and (nonlinearity) into the group deter- ining birth weight.

We wish, however, to get some idea of le error involved in ignoring the possible fluence of fetal growth rate on the gesta- on period. To do this we may borrow an dditional equation from Ibsen's data by aking a direct estimate of p_{BA} (or b_2). he mean rate of gain near the end of station in Ibsen's stock ($\overline{F}_I$) is given by e regression of weight on fetal age $C_{BA(I)}$) in his data. The expected birth eights on the basis of the observed litter

TABLE 7

Path coefficients relating to birth weight estimated from correlations in table 5 and (in parentheses) assumptions or estimates based on Ibsen's data

	B 1910–15			B 1916–18			A 1906–15		
	1	2	3	1	2	3	1	2	3
r_{ES}	(0)	(0)	(0.500)	(0)	(0)	(0.500)	(0)	(0)	(0.500)
$f_1 = p_{FL}$	−0.658	−0.646	−0.749	−0.641	−0.606	−0.731	−0.646	−0.663	−0.744
$f_2 = p_{FS}$	+0.753	+0.763	+0.663	+0.767	+0.796	+0.682	+0.763	+0.749	+0.668
$a_1 = p_{AL}$	−0.526	−0.556	−0.526	−0.501	−0.581	−0.501	−0.474	−0.414	−0.474
$a_2 = p_{AF}$	(0)	−0.046	(0)	(0)	−0.132	(0)	(0)	+0.090	(0)
$a_3 = p_{AU}$	+0.850	+0.850	+0.850	+0.865	+0.859	+0.865	+0.880	+0.892	+0.880
$b_1 = p_{BF}$	+0.824	+0.814	+0.724	+0.833	+0.808	+0.730	+0.761	+0.778	+0.661
$b_2 = p_{BA}$	+0.249	(+0.279)	+0.249	+0.243	(+0.333)	+0.243	+0.366	(+0.314)	+0.366
$b_3 = p_{BE}$	(+0.322)	(+0.322)	(+0.322)	(+0.322)	(+0.322)	(+0.322)	(+0.322)	(+0.322)	(+0.322)
$b_4 = p_{BN}$	(+0.115)	(+0.115)	(+0.115)	(+0.115)	(+0.115)	(+0.115)	(+0.115)	(+0.115)	(+0.115)

TABLE 8

	B 1910–15	B 1916–18	A 1906–15
$p_{BFL}=b_1f_1=$	−0.542	−0.534	−0.492
$p_{BAL}=b_2a_1=$	−0.131	−0.122	−0.173
r_{BL} =	−0.673	−0.656	−0.665

sizes ($\overline{L}$) and intervals ($\overline{I}$) in our three sets of data can be calculated by the formula given earlier. They came out considerably larger than observed in these sets indicating that Ibsen's stock was heavier. The ratio $\overline{B}/\overline{B}_I$ may be applied to the values of $C_{BA(I)}$ to obtain an estimate, C_{BA}, for our data. An estimate of the standard deviation of fetal age at birth (σ_A) can be obtained from the observed standard deviation of intervals by the formula $0.95\sigma_I$. The path coefficient p_{BA} is given by the formula $p_{BA} = C_{BA}\ \sigma_B/\sigma_A$ (table 9).

These estimates permit determinate solution on introduction of another path. That relating gestation period to late fetal growth rate (coefficient p_{AF}) seems of most interest, ignoring the probably much smaller effect of variation in the early part of the growth curve (p_{AE}). An alternative would be to treat birth weight and gestation period as acting reciprocally as suggested by Tukey ('54) by introducing a coefficient p_{AB}. This, however, complicates considerably the analysis. It may suffice here to attribute the whole readjustment, required by estimating p_{BA} on the basis of Ibsen's results, to p_{AF} recognizing that this tends to exaggerate the latter slightly. The new sets of equations are easily solved.

$r_{BL} = b_1f_1 + b_2r_{AL}$
from which $b_1f_1 = r_{BL} - b_2r_{AL}$

$r_{BA} = b_1r_{AF} + b_2$
from which $b_1r_{AF} = r_{BA} - b_2$

$r_{BB} = 1 = b_1^2 + b_2^2 + b_3^2 + b_4^2 + 2b_1b_2r_{AF}$
solve for b_1

$f_1 = (r_{BL} - b_2r_{AL})/b_1$

$r_{AF} = (r_{BA} - b_2)/b_1 = a_1f_1 + a_2$
$r_{AL} = \quad a_1 + a_2f_1$
solve for a_1 and a_2

$r_{AA} = 1 = a_1^2 + a_2^2 + a_3^2 + 2a_1a_2f_1$
solve for a_3

The solutions are given in table 7 the second columns of each set. The ana ysis of r_{BL} is as shown in table 10.

The estimates of p_{AF} are small and i consistent and the analysis of the correl tion between birth weight and size of litt is not modified to an extent that can l considered as of any importance. Th introduction of a path measured by p could not change these results appreciabl So far it appears that time of parturitio in influenced considerably by the numb in a litter but very little if any of the si of the fetuses.

We have not, however, taken a count of the positive correlations tha certainly exist to some extent b tween the ultimate growth factors and S and probably also between each o

TABLE 9

	Estimates from Ibsen's data					
	$C_{BA(I)}$	$\overline{B}_I$	$\overline{B}/\overline{B}_I$	C_{BA}	σ_A	p_{BA}
Control (1910–15)	3.64	98.0	0.839	3.05	1.79	0.279
Control (1916–18)	4.09	102.9	0.761	3.11	1.97	0.332
Inbred (1906–15)	3.81	98.1	0.846	3.22	1.78	0.318

TABLE 10

	B 1910–15	B 1916–18	A 1906–15
$p_{BFL}=b_1f_1$ =	−0.526	−0.490	−0.516
$p_{BAL}=b_2a_1$ =	−0.155	−0.193	−0.130
$p_{BAFL}=b_2a_2f_1=$	+0.008	+0.027	−0.019
r_{BL} =	−0.673	−0.656	−0.665

ese and size of litter (L). Unfortunately e available data do not permit a solution. 'e may, however, test the effect of arbi- arily assuming a moderately large corre- tion (+ 0.50) between the two growth fac- rs. It will be assumed that r_{LE}, r_{LS} as well s p_{AF} are negligible. The solutions are ven in the third columns under each set table 7. There is considerable readjust- ent, but the components of the correla- ons between birth weight and litter size e the same as in the earlier solution in hich p_{AF} was ignored.

We may, indeed, assume any values hatever for r_{ES}, r_{LE} and r_{LS} without affect- g the values of p_{BA}, p_{AL} and the resulting ontribution of litter size to birth weight y way of gestation period ($p_{BAL} = p_{BA}p_{AL}$) s long as p_{AF} is assumed to be negligible. here is, of course, considerable readjust- ent among the coefficients by which birth eight is related to litter size in other ays, but the total for the compound sidual path $p_{(BL)}$ is necessarily unaffected. hus if $r_{ES} = 0.50$, $r_{LE} = 0.30$, $r_{LS} = 0$, we ave the analysis, shown in table 11, of the ontribution to r_{BL}.

The gain to 33 days is represented in gure 7 as affected by perinatal mortality M), size of litter (L), gestation period A), birth weight (B) and residual factors T). The direct effect of perinatal mortal- y is positive because of the effect on com- etition. Perinatal mortality itself should e affected positively by the direct effect f litter size (large litters, heavy mortality), egatively by the direct effect of A (pre- ature birth, heavy mortality), and nega- vely by the direct effect of B (excessive ze at birth, heavy mortality from diffi- lty in parturition and anoxia). The rect effect of L on G (large litters, severe mpetition) should be opposite in sign to s indirect effect through M. Similarly the rect effect of A on G (premature birth, ow gain) should be opposite in sign to its indirect effect through M. The direct effect of B on G (large amount of growing mate- rial, large gains) should, on the other hand, reinforce the indirect effect through M. The effect of L, A, and B on M are probably far from linear (important only for large L, for small A, and for large B), thereby accounting for the highly nonlinear total effect of L on M (litters of two or three optimal). It thus seems best to com- bine the effects through M with the direct effects and not include M explicitly in the analysis.

This leaves G a function of L, A, B and the residual factor T. If the latter is treated as an independent variable, the path coeffi- cients relating G to its factors become ordinary abstract partial regression coeffi- cients. Using the symbols of figure 7 and table 7 we have the following equations.

$$r_{GL} = g_1 + r_{AL}g_2 + r_{BL}g_3$$
$$r_{GA} = r_{AL}g_1 + g_2 + r_{BA}g_3$$
$$r_{GB} = r_{BL}g_1 + r_{BA}g_2 + g_3$$
$$r_{GG} = 1 = g_1r_{GL} + g_2r_{GA} + g_3r_{GB} + g_4^2$$

The solutions are given in the first colu- ums under the three sets in table 12.

According to these solutions, gain is affected considerably by birth weight in all cases, but the effects of litter size and of gestation period are small and incon- sistent. This is not wholly unreasonable in view of the opposition between the direct effects of L and A and their indirect effects through M. We have, however, taken no cognizance of the positive correla- tions that almost certainly exist among the residual factors for all growth processes. Recognition of these tends to subtract from the effect attributed directly to birth weight and to increase the effects attributed to L and A.

As an extreme hypothesis, it will be as- sumed that $r_{ES} = 0.500$ and that r_{ET} and r_{ST} are equal and that there is no direct effect of birth weight or indirect effect through M ($g_3 = 0$). A term g_4r_{BT} is added

TABLE 11

	B 1910–15	B 1916–18	A 1906–15
$p_{BFL} = b_1f_1$	−0.639	−0.631	−0.588
$p_{BEL} = b_3r_{LE}$	+0.097	+0.097	+0.097
$p_{BAL} = b_2a_1$	−0.131	−0.122	−0.173
r_{BL}	−0.673	−0.656	−0.665

TABLE 12

Path coefficients, relating to gain to 33 days, estimated from correlations in table 5 and assumptions (in parentheses)

	B 1910–15			B 1916–18		A 1906–15		
	$r_{BT} = 0$	$g_3 = g_4 r_{BT}$	$g_3 = 0$	$r_{BT} = 0$	$g_3 = g_4 r_{BT}$	$g_3 = 0$	$r_{BT} = 0$	$g_3 = g_4 r_{BT}$	$g_3 = 0$
r_{ES}	—	(0.500)	(0.500)	—	(0.500)	(0.500)	—	(0.500)	(0.500)
r_{ET}	(0)	(0.209)	(0.303)	(0)	(0.248)	(0.360)	(0)	(0.264)	(0.366)
r_{ST}	(0)	(0.209)	(0.303)	(0)	(0.248)	(0.360)	(0)	(0.264)	(0.366)
r_{BT}	(0)	(0.167)	(0.243)	(0)	(0.203)	(0.295)	(0)	(0.201)	(0.279)
$g_1 = p_{GL}$	−0.085	−0.238	−0.315	−0.224	−0.391	−0.479	+0.002	−0.191	−0.279
$g_2 = p_{GA}$	+0.129	+0.199	+0.234	−0.034	+0.042	+0.082	−0.065	+0.078	+0.144
$g_3 = p_{GB}$	+0.424	+0.142	(0)	+0.478	+0.164	(0)	+0.571	+0.178	(0)
$g_4 = p_{GT}$	+0.823	+0.847	+0.877	+0.778	+0.810	+0.851	+0.846	+0.886	+0.929
$w_1 = p_{WB}$	+0.284	+0.284	+0.284	+0.338	+0.338	+0.338	+0.315	+0.315	+0.315
$w_2 = p_{WG}$	+0.815	+0.815	+0.815	+0.758	+0.758	+0.758	+0.796	+0.796	+0.796

to the equation for r_{GB} in the preceding equations ($r_{BT} = b_1 f_2 r_{ST} + b_3 r_{ET}$). The new equations yield the results shown in the third columns in table 12 under the various sets. These give fairly consistent and reasonable results as far as the effects of L and A are concerned but are probably too extreme in allowing no direct influence of birth weight (including here the reinforcement from the effect through M). An intermediate result can be obtained by putting the direct influence measured by equal to the indirect one through T ($g_4 r_{BT}$). In this case the term g_4^2 in the equation for r_{GG} must be replaced by $g_4\, r_{GT}$ in which $r_{GT} = g_4 + g_3 r_{BT}$. The solutions are given in the second columns in table 12 for the various sets. These probably give a more satisfactory interpretation than either of the preceding. Those under B (1910–15) are used in figure 7 together with those in the third columns of table 7.

Weight at 33 days (W) is merely the sum of the birth weight and gain to 33 days. The correlations involving W were calculated from the observed variances of B and G and the correlation r_{GB}.

$$\sigma_W^2 = \sigma_B^2 + \sigma_G^2 + 2\sigma_B\sigma_G r_{GB}$$
$$p_{WB} = \sigma_B/\sigma_W, p_{WG} = \sigma_G/\sigma_W$$
$$r_{WX} = p_{WB} r_{BX} + p_{WG} r_{GX}$$

where X is any other variable.

The path diagram gives a picture of the network of relations among the perinatal characters in so far as quantitative evaluation has been possible from the data at hand. It is, of course, only a partial picture. Perinatal mortality (M) is introduced only qualitatively. In a more complete qualitative scheme, M should be analyzed at least into mortality at or before birth and mortality between birth and weaning since, as noted, these are determined to a considerable extent by different factors. Size of litter (L) could be analyzed into amounts of ovulation, percentage of implantation and percentage surviving early death and absorption. These are affected by genetic factors of the dam and of the individual and through the condition of the dam by external environment factors. The interval since the preceding litter (more or less than 76 days) is positively related to following and perhaps also preceding litter size. Amount of ovulatio

correlated with the growth heredity of e dam. The various growth factors (E, T) involve the dam's heredity and that the individual in different degrees, and spects of the condition of the dam, that ace to the external environments at suc- ssive times.

CONCLUSIONS

The most dramatic of the vital char- cteristics of the guinea pig colony under nsideration have been the malforma- ons that lead inevitably to death at some age of development or to increased nances of death. There have been a mited number of elementary abnormal- ies visible without dissection: for exam- le, cruciate doubling of the whole body, a roup tracing to early anterior inhibition such as defects of mandible, premaxil- ry, maxillaries, nose, cyclopean eye), notia, microphthalmia, hydrocephalus nd exencephalus, cleft palate and harelip, efects of the limb buds (atavistic little e, thumb, and big toe, ventral flexure of e feet, torsion of the legs, micromelia, normalities of the digits), abnormality the axial skeleton, dropsy, anemia, re- uced sterile testes.

Analysis of matings in which these have peared indicate that most of them re- uire the conjunction of deviant heredity nd unfavorable environmental stress, that e same elementary abnormality may ace to different heredities, and that dif- rent ones may be induced alternatively in various combinations by closely simi- r heredities and environmental condi- ons. In some cases, elementary abnor- alities may be compounded in rather efinite sequence with increasing stress. other cases, there is much irregularity. he specificity of a particular elementary normality resides primarily in the sus- ptibility of a particular developmental rocess to inhibition whether by unfavor- le gene products or unfavorable environ- ent.

The most characteristic genetic basis for orphological abnormality seems to be the mulative physiological action of multiple nes and a threshold at which homeo- atic control of normal development eaks down. In some cases, however, a ngle gene (e.g., *Px*) or particular com- bination of genes (e.g., *sisidmdm*) brings about such a drastic disturbance that a certain complex of abnormalities is de- termined with considerable regularity. Even in these, some elements of the syn- drome usually show irregular penetrance.

It is an interesting question whether all morphological evolution depends on genes with effects at the morphological level as nonspecific as those considered here (cf. Wright and Eaton, '23; Wright, '34b). The contrast between the wild array of unre- lated abnormalities brought about in *Px/Px* and the approach to a once normal mor- phological pattern of the feet brought about by the same gene in one dose suggest that genes of these sorts may be utilized in evolution through enhancement of adap- tive effects and buffering against unfavor- able ones in suitable combinations with other genes.

The significance of the array of malfor- mations in the vital statistics of the guinea pig was not very great. The otocephaly of one strain (number 13) played a signifi- cant role in its percentage of still births, but this was in lines deliberately expanded for study. The typical rate of occurrence in the colony in general was about 0.04%. Monstrous polydactyly must be left out of consideration since this traced wholly to a single mutation. The commonest simple abnormality was microphthalmia (0.19%). Ventral flexure of the feet and torsion of the limbs (excluding that associated with the chunky monstrosity) accounted for 0.07%. The chunky type accounted for 0.03%. Hydrocephalus and exencephalus also accounted for 0.03%. Miscellaneous defects of digits, face abnormalities, exom- phalus, and others accounted for 0.04% The grand total of those merely recorded as they occurred without selection is thus about 0.4%.

Genetic differences not associated with morphological defect played a much more important role in the vital statistics of the colony than those that were associated with such defects. Marked differences in frequency and size of litter, in percentages born alive and reared of those born alive and in birth weights and early gains came to be characteristic of different inbred strains and were undoubtedly segregating within the control random bred stock. Like

the morphological deviants, all these characters are profoundly affected by environmental conditions as well as by heredity. Because of the network of interactions the genetic factors may all be expected to have pleiotropic effects on all the vital characters.

OPEN DISCUSSION

E. S. RUSSELL[4]: I would like to tell people that, in case they want the stock, and have facilities for handling the animals, I have this very interesting case of the combination of two recessive genes making a specific type of pleiotropism—that is, the anemia, pigment-loss, and sterility syndrome in the silver-diminished guinea pig. I have a small stock of these; it may be a stock value in some other ways. I know that it contains *c* and *p*. I don't know whether the fancy has all these guinea pig genes, but the thing that is particularly valuable in the stock is that all the animals are silver and they are segregating for diminished. I have tested pairs of heterozygotes and some sterile silver-diminished males and some silver-diminished females, some of which show limited fertility.

If anybody is interested in acquiring a couple of pairs of tested heterozygotes, I would be delighted to supply them. I hope that somebody will explore this particular problem further.

WRIGHT: I don't have any of these now.

E. S. RUSSELL: I think probably I have the only ones that there are. Eventually I may be able to do something with them myself, but it is to accelerate the progress of science.

OWEN[5]: Dr. Wright, in one or two of your slides you assigned a sex difference in the incidence of certain characteristics to the X chromosome. I supose that we mammalian geneticists might well note a recent proud possession—a Y chromosome with some character to it. Could your data as well be interpreted as effects of the Y?

WRIGHT: That is certainly a possibility. The marked sex difference in frequency could be due to the Y chromosome as well as to the X. It is not due to differential prenatal mortality and can hardly be due to sex hormones because of the earliness of determination. I have noted that the microphthalmics were also twice as fr quently female as male but this was n true of the rest of the abnormalities.

OWEN: In your long experience, ha you ever noted any specific pedigree indic tion of differences among Y's in the effects on characters of this sort?

WRIGHT: No.

WAELSCH[6]: Was there any absence pigment in this family?

WRIGHT: This family was almost blac eyed white. The median percentage w 97.0 white in males, 98.6 in femal in line with the usual sex difference. was homozygous silver (*si/si*) as well spotted (*s/s*).

BATEMAN[7]: Do guinea pigs never e their young, however malformed they are

WRIGHT: If the young are born dea the mother occasionally chews on them little. Guinea pigs are rather big thing at birth. A still born singleton may weig 150 g and the mother perhaps only 60 g, four times as heavy. It would be rather big meal. The newborn is covere with fur and so perhaps is not as attractiv a thing to eat as a newborn mouse c rabbit.

BATEMAN: What do you think is th reason that you are getting these guine pigs that are so abnormal?

WRIGHT: One gets these, I think, in an stock of animals that is studied lon enough. Among our 120,000 records, onl about 0.4% were abnormal, including rela tively minor abnormalities other than pres ence of a little toe, but excluding type that were deliberately multiplied. Flexur of the palm is one of the commoner non lethal types. An animal with this defec does fairly well but has to walk on it knuckles.

BATEMAN: It was the monsters I wa thinking of.

WRIGHT: Certain of these were multi plied deliberately. The polydactyl mon sters, the most extreme type, descende from one mutant individual. Several thou

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[4] E. S. Russell, Roscoe B. Jackson Memorial Laboratory.

[5] R. D. Owen, California Institute of Technology.

[6] S. G. Waelsch, Albert Einstein College of Medicine.

[7] A. J. Bateman, Christie Hospital, Manchester

ınd heterozygotes were produced, largely ı linkage studies. Ninety-two per cent of ıe monstrous homozygotes die and are bsorbed in early gestation, yet I saw 79 f them. The fact that I recorded over 500 tocephalic monsters was due largely to eliberate multiplication of strain 13, hich for over a quarter of a century pro- uced nearly 5% in all branches but one hich produced 28% in one big branch.

SILVERS[8]: I just wonder whether dis- rganization in the mouse is anything simi- ar to this. Dr. Hummel, you do get a ituation in which you seem to get all kinds f monsters with or without being able to redict what is going to happen, though, on't you?

HUMMEL[9]: I think certainly it must be metabolic defect, but what causes the veaknesses at certain points of course is he question.

WRIGHT: There are certain types of bnormal development that seem well ocalized in time and space; for example, he atavistic little toe of strain D. In thers, the overt effect of gene action eems much less specific. Any sort of eneral disturbance at the right time may ring out microphthalmia. Probably many lifferent genes and many sorts of environ- nental disturbance will bring it out.

PAPAZIAN[10]: You mentioned that abnor- nalities, in particular polydactyly, may epresent weak or unstable pathways in levelopment. You also said that poly- lactyly was atavistic. These thoughts to- ether suggest that more recently evolved haracters, owing to more recently incor- orated pathways, are less stable and there- ore more easily destroyed. Moreover, the lestruction or blocking of a recently in- orporated pathway would not be lethal but vould leave the organism with its an- estral, balanced, set of pathways.

WRIGHT: The perfectly normal-seem- ng thumb, little toe, and big toe found nore or less regularly with genotype *?x/px* suggest highly specific effects in he same category as the little toe brought bout by multiple factors. Yet the homo- ygote, *Px/Px,* produces a grossly abnor- nal foot with a dozen digits or so and an xtraordinary array of other abnormalities n almost all parts of the body. We may uppose that the atavistic type of foot is caused merely by a weakness in the developmental process that leads to suppression of the normal suppressors of the little toe, etc. The heredity for development of the little toe, the thumb, and big toe are all deeply imbedded in the genetic complex of the guinea pig as a result of the millions of years in which the pentadactyl foot was characteristic of mammals and their ancestors. The suppression of these digits, a process like the suppression of the lateral digits of the horse, has been superimposed on this developmental pattern in only the last few million years. There could hardly be a simple dropping out of genes that stand for little toe, thumb, and big toe. The suppression may be brought about by a narrowing of the limb bud or by a premature differentiation before all the normal lobes have appeared. Any sort of disturbance, genetic or environmental, that interferes with this relatively recently acquired process tends to bring back the old pentadactyl foot.

It is an interesting evolutionary question whether orderly evolution can come out of mutations with such irregular unspecific effect as suggested for these guinea pig abnormalities. Perhaps by gradually putting together the right assemblage of modifiers to give a good deal of buffering and double assurance, a smooth orderly developmental result can emerge from such apparently unpromising genetic material.

SEARLE[11]: Dr. Wright showed some curves giving the results of crosses between strains of guinea pigs with different degrees of polydactyly. As far as I could see, in the F_1 and even in the F_2 between these strains, the variability with regard to this threshold character was less than in the pure line. Was this actually a fact?

WRIGHT: The F_2 variability was much greater. Of course these curves have varying degrees of validity. No estimate of variability could be obtained from strains that were wholly 3-toed or wholly 4-toed, All three phenotypes were, however, pres-

[8] W. K. Silvers, The Wistar Institute of Anatomy and Biology.

[9] K. P. Hummel, Roscoe B. Jackson Memorial Laboratory.

[10] H. Papazian, University of Connecticut, Storrs.

[11] A. G. Searle, Radiobiological Research Unit, Harwell, Berkshire.

ent in strain 35, even in a large branch that was derived wholly from a single mating in the twenty-second generation of brother-sister mating and that showed no correlation between parents and offspring. Taking the interval between the thresholds as the unit on an underlying physiological scale and assuming normal variability, a standard deviation of about 0.80 was indicated. This value was then taken as a measure of the variability from nongenetic factors (principally age of mother and season of birth) and applied to all inbred strains and their first crosses.

The mean of strain D (100% good 4-toed) was taken as at + 3.00 relative to the lower threshold (2.5σ above the upper threshold). If lower, one would expect some poor 4-toed offspring. It could not have been much higher, since selection could hardly carry the mean beyond the point at which substantially all animals were good 4-toed. Similarly the mean of F_1 (2 × D) in which all 146 animals were 3-toed is located at − 2.0 (2.5 σ below the lower threshold). The absence of even poor 4-toed young indicates that it could not have been appreciably higher, but the production of a poor 4-toed in 26 F_1's from the cross of strain D with the 3-toed strain 32, which gave results in F_2, and the backcross to D, which did not differ significantly from those derived from 2 × D, indicate that the mean of F_1 (2 × D) could not have been much lower. The mean of strain 2 itself (3-toed) is located at − 7.00 on the hypothesis that F_1 is exactly intermediate.

All three phenotypes were found in reasonably high frequencies in F_2, the backcross to D, and in tests of the apparent 3-toed and 4-toed segregants in the backcross made by a second backcross to D. A unique normal curve is thus determined by each of these trichotomies, and means and standard deviations can be calculated as shown in figure 1. Third backcrosses to D were also carried through in moderately large numbers. It is to be noted that the means shift systematically toward D and that is also true of the standard deviations.

The results in F_2 and the first backcross can be accounted for on the hypothesis that there are about four equivalent factors differentiating F_1 and from D. They can also be accounted for on the hypothes that there is one leading factor that a counts for about half this difference a a multiplicity of minor ones or by inte mediate hypotheses (e.g., a series of fa tors with effects in geometric series su that the leading factor accounts for 40 of the total difference, the second 24 the third 14.4% and so on). The succe of continued selection for 3-toe in preven ing further approach of the mean to th of D in a third backcross to the latt (young 15/16 D) suggest fixation at th point for all but one or two leading factor The reduction of the standard deviatio practically to that of an inbred strain i dicates that 3-toe at this point can hard depend on less than two factors. Thus a intermediate hypothesis similar to the or cited last seems indicated.

As I have noted, the rather close simul tion in this case to one factor segregatio in terms of 3-toe versus 4-toe of any grad in F_2 and the backcross broke down o testing the supposed segregants in th backcross (and also in F_2). Other crosse (13 × D) gave no such simulation of on factor heredity but gave results in F_2 an the backcross that were fully in harmon with the hypothesis of multiple factors an two thresholds with respect to an under lying physiological scale.

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The Genetics of Litter Size in Mice

D. S. FALCONER
Agricultural Research Council Unit of Animal Genetics, Institute of Animal Genetics, Edinburgh

Litter size is a quantitative character of some considerable complexity, and the title of this paper should more properly have been "Some aspects of the genetics of litter size." The complexity arises mainly from the fact that the character belongs partly to the parental generation and partly to the filial generation; that is to say, the number of young born in a litter depends partly on the fertility of the parents—chiefly, as we shall see, that of the female—and partly on the viability of the embryos that will constitute the litter. There is also an interesting maternal effect, but its interest hardly compensates for the difficulties it introduces. For these reasons a complete description of the genetics of litter size is a goal for the future rather than a present achievement.

In this paper I shall give an outline of a series of investigations made by R. C. Roberts, J. C. Bowman, and myself, which were concerned principally with the reactions of litter size to inbreeding and to artificial selection, and with the nature of the changes produced by these two procedures. From the reaction to inbreeding we can learn something about the dominance relations of the genes that influence litter size, and from the response to selection we can determine the proportionate amount of additive genetic variance. The total amount of genetic variance can be discovered only from a comparison of the variances of genetically uniform and genetically heterogeneous groups, and this has not yet been done. In addition to the inbreeding and selection, some studies were also made on an unselected control population, from which information was obtained about the influence of male fertility, the maternal effect, and the parent-offspring correlation. The investigations of the nature of the changes produced by inbreeding and by selection were aimed at discovering the extent to which ovulation rate, implantation rate, and fetal mortality were involved. A preliminary account of the selection experiment was published some time ago (Falconer, '55). Descriptions of the inbreeding experiments will be found in three papers, by Roberts ('60), Bowman and Falconer ('60), and Falconer and Roberts ('60). The remaining work summarized here will be fully described elsewhere.

For the purposes of measurement in all the experiments, litter size was taken to be the number of live young found in the first litters of females aged between 6 and about 9 weeks. All the experiments were done on the same basic stock, known in the laboratory as the J stock. It originated in crosses between several different non-inbred strains and had subsequently been maintained by random mating for some ten generations.

INBREEDING

Inbreeding depression. Litter size, as everybody knows, is reduced by inbreeding. The conclusion to be drawn is that the genes that reduce litter size are on the average recessive to their alleles that increase it. Figure 1 shows the rate of decline found with intense inbreeding, and very little selection, in two experiments. In the first experiment (upper graph) there were thirty lines inbred by full-sib matings with no artificial selection. In the second experiment (lower graph) there were 20 lines inbred by a double-first-cousin mating followed by consecutive full-sib matings. Selection within lines was applied to ten of the lines, but the intensity of selection was low and the rate of decline was not affected. The two groups of lines are not shown separately on the graph. The decline of litter size in both experiments was linear with re-

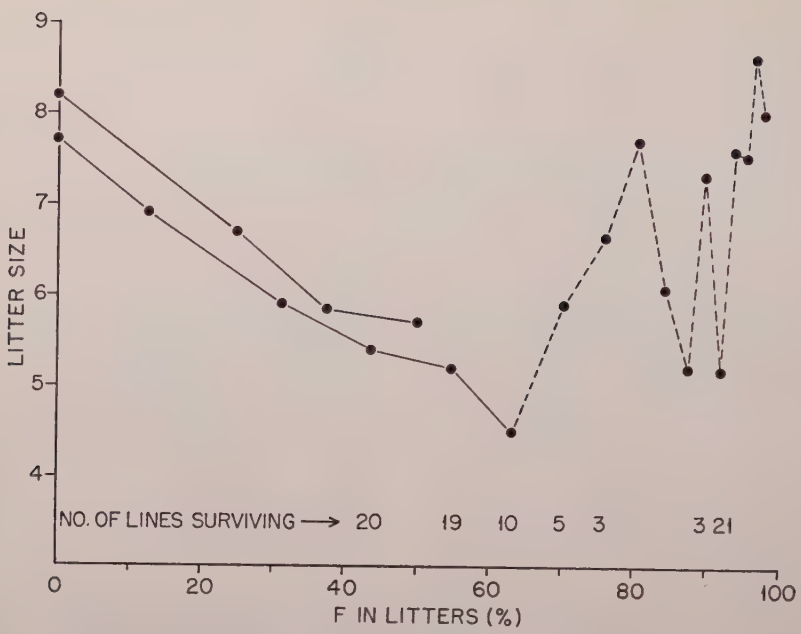

Fig. 1 Reaction of litter size to rapid inbreeding. Mean litter size plotted against the inbreeding coefficient of the litters.

spect to the coefficient of inbreeding, and the rate was 0.49 young per 10% increase of inbreeding in the first experiment, and 0.56 young in the second experiment. If the linear decline had continued indefinitely the litter size would have been reduced to two young at 100% inbreeding. The first experiment was stopped after three sib matings ($F = 0.5$), and nothing further will be said about it here. The second experiment was continued for as long as the lines survived. Each line was propagated from the first litter of one female. All the offspring in this litter were mated and the line was continued from one of them. Any line became extinct when there was no litter containing at least one of each sex. All of the 20 lines survived to an inbreeding coefficient of 44%. Three-quarters of the lines were lost in the next three generations, and by the time the inbreeding coefficient had reached 76% only three lines survived. The loss of lines resulted in an increase of the mean litter size, because, of course, the surviving lines were those with the higher litter sizes. Two of the three surviving lines dropped out at the eleventh and twelfth generations when the inbreeding coefficient was about 90%. The remaining one out of the original 20 lines survived indefinitely and its mean litter size was equal to, or a little above, the noninbred controls. (This line, now in its twenty-eighth generation, has the official status of an "inbred strain" and is known as JU.)

The records of the three lines that survived longest showed that these lines were not particularly good ones at the beginning, and their long survival was due to the fact that they did not decline in litter size. This absence of inbreeding depression in some lines, and the fact that one line reached very high levels of inbreeding without any decline of litter size proves, I think, that overdominance cannot have been a major factor in the inbreeding depression of this population. In other words, there cannot have been any overdominant locus with more than a trivial effect on litter size. Simple dominance—or deleterious recessives—is a perfectly adequate explanation of the situation. From the practical point of view the results of this experiment show that selection between lines is effective in counteracting the inbreeding depression of litter size, and that

one aims to end up with a certain num-
r of highly inbred lines one must have
mewhere about 20 times this number
the start.

Relative importance of inbreeding in other and young. The effect of inbreed-
g on litter size is complicated by the
ct that, under continuous inbreeding,
e inbreeding coefficient of the young in
e litters is always one step ahead of that
the mothers. The reduction of litter
ze may be due partly to the reduced
rtility of the females and partly to the
duced viability of the embryos. In order
separate the effects of inbreeding on
e young from those on the mother,
osses were made between partly inbred
nes and the litter sizes of the inbred
others with crossbred young were com-
ared with those of equally inbred
others with inbred young. The mean
tter sizes found are given in table 1.
he three comparisons in the first row
ow the effect of inbreeding on the fer-
lity of the mothers, and they give a mean
lue of 0.175 for the reduction of litter
ze per 10% of inbreeding. The compari-
sons in the second and third columns show the effect of inbreeding on the viability of the young, and they give a mean value of 0.245 for the reduction per 10% of inbreeding. Adding the two contributions together gives about 0.42 young which agrees well enough with the rate of decline under continuous inbreeding. About 40% of the total inbreeding depression of litter size is thus attributable to reduced fertility of the females and about 60% to reduced viability of the young. The inbreeding of the father, it should be added, did not influence the size of the litter sired.

Cause of reduced fertility. The reduced viability of the inbred embryos was not further investigated, though for the sake of completeness it would be interesting to know the developmental stage at which death most frequently occurs. The cause of the reduced fertility of inbred mothers was, however, investigated by dissections of pregnant females. Inbred females were mated to males of another line so that the embryos were noninbred. Dissections were made at 16 days of gestation, counted from the finding of a vaginal plug. Counts

TABLE 1

Mean litter sizes according to the inbreeding coefficients of the mothers and of the young

		Inbreeding coefficient of mothers		
		0%	37½%	50%
		mean no. of young per litter		
Inbreeding coefficient of young	0%	8.2	7.5	7.3
	50%	—	6.3	—
	59%	—	—	5.8

Reduction of litter size per 10% inbreeding of { mother: 0.19, 0.18, 0.16. young: 0.24, 0.25.

TABLE 2

Numbers of corpora lutea and percentage losses in inbred and noninbred females

Females dissected			Mean no. of corpora lutea	Mean loss		
Series	Inbreeding coefficient	No.		Preimplantation: % of corpora lutea	Postimplantation: % of implants	Total: % of corpora lutea
	%					
I	50–59	86	10.0	17.6	12.9	28.4
	0	58	10.1	11.1	11.5	21.4
II	50	13	10.9	22.5	13.6	33.1
	0	15	11.7	4.0	13.0	16.6
III	63	17	12.5	37.1	18.7	49.0
	0	59	10.3	12.0	10.2	21.0

were made of the corpora lutea (as a measure of the ovulation rate), the numbers of implantation sites, and the numbers of live embryos. These counts were then compared with similar counts made on comparable noninbred females. The results are summarized in table 2. Series I and III refer to the first and second inbreeding experiments described here, and series II refers to another inbreeding experiment with the same stock, which also provided the data for table 1. The results show clearly that the reduced fertility of the inbred females was due almost entirely to a greater preimplantation loss of eggs or embryos. The ovulation rate was not reduced, and the postimplantation loss was only a little, and non-significantly, increased. The preimplantation loss was, however, much greater in inbred than noninbred females, and the differences are significant at the 5% level in series I and II and at the 1% level in series III. Three possible causes of the greater preimplantation losses in inbred females may be postulated, but I do not know which is the right one. One cause might be a higher proportion of abnormal eggs; another might be failure of fertilization through impaired transport of the sperm; and the third might be failure of implantation from endocrine malfunction.

The fact that the ovulation rate was not affected by inbreeding calls for some comment. Ovulation rate is correlated with body size; the regression of the number of corpora lutea on the weight of the female at 6 weeks was 0.24 ± 0.06 corpora lutea per gram. Body size might well be expected to decline on inbreeding and, so to speak, carry the ovulation rate with it. But in fact the body size of the mice i these experiments did not decline on in breeding, because the reduction of litte size led to an improved maternal enviro ment which compensated for any declin of intrinsic growth rate that there ma have been. Thus the conclusion that th ovulation rate is independent of inbree ing is valid only if there is no change o body size. The conclusion about gene a tion that we can draw is that the gene that affect ovulation rate independentl of body size do not show directional dom inance, though the genes that affect through their effects on body size ma do so.

THE CONTROL LINE

An unselected control line was main tained with minimal inbreeding over th whole course of the inbreeding and selec tion experiments, and there are some con clusions to be drawn from it that shoul be described before we consider the selec tion.

Inbreeding in the control line. The con trol line was maintained by ten pairs o parents in each generation, with equa representation among the parents of th next. The effective population size wa therefore 40, and the rate of inbreedin was 1.25% per generation. The litter siz in the control line did not change system atically and, apart from irregular fluctua tions, it remained at about 7.5 young ove the whole course of the experiment. Thi fact (which may be seen from figure 2) besides being very convenient for th analysis of the selection responses, is als interesting in connection with the inbreed ing. The inbreeding coefficient computed from the effective population size work

TABLE 3

Analysis of variance of litter size in the control line up to generation 28

Source of variation	d.f.	M.S.	Variance component
Between generations	27	7.64[a]	0.11
Within generations	882	4.11	4.11

Variance of observed generation means

Expected			Observed
Real	Sampling	Total	
0.11	0.13	0.24	0.23

[a] $F = 1.86$; $P < 0.01$. Mean number of litters per generation, 32.5.

ut to be 32% at generation 31. If the itter size had declined at the same rate s it did with rapid inbreeding the con- rol line would have dropped to a mean of bout six young by the end. A decline of his amount would certainly have been letected. It therefore looks as if natural election has been effective in counteract- ng the inbreeding. If natural selection is ndeed the explanation, then it must have vorked chiefly through its action on the iability of the young because, on account f the breeding system, there was very ittle opportunity for it to act on the fer- ility of the female. There may, however, e no need to invoke natural selection be- ause, it will be remembered, three of the 0 rapidly inbred lines reached much igher levels of inbreeding without show- ng any decline of litter size. Whatever nay be its real explanation, the constancy f the control line suggests that an effec- ive population size of 40 may be large nough to allow a strain to be maintained or many generations without any dete- ioration of litter size.

Variation between generations. From he graph of the control line in figure 2 it will be seen that the mean litter size fluc- uated eratically between the limits of 6.9 nd 8.6. The variance of the observed eneration means is 0.23. How much of his variation between the generations was eal and how much due to sampling? his question was answered by a simple nalysis of variance between and within enerations up to generation 28, which is hown in table 3. The variation between enerations is significant at the 1% level nd therefore without doubt is real. But it s rather small in amount: the component etween generations is only some 2½% f the variance within generations. When he possible sources of variation between enerations are considered—quality of the ood, temperature, light, and other sea- onal effects, it does seem surprising that hese have so little influence on litter size n comparison with the differences be- ween contemporaneous individuals. This, owever, does not answer the question of ow much of the observed variation is real. he variance of the observed generation eans is the sum of the real variance, hich is 0.11 (from table 3), and the sampling variance. The expected sampling variance is $1/n$ times the within-generation variance, where n is the number of litters per generation, and this works out to be 0.13. Thus about half of the variance that appears as fluctuations of the generation means is attributable to sampling and half to real differences between the generations.

Variation attributable to male fertility. The records of the control line provide information about the effect of the male on the size of the litter he sires. It is necessary first to explain what the records consisted of. Each generation consisted of the first-litter progeny of ten pairs of parents. These ten full-sib families contained, on the average, about three or four females. Each of these females was test-mated to a male from another family, and her litter size was recorded from the litter subsequently born. Before the test litters were born one female of each sibship was chosen to be a parent, so that her litter was retained for testing in the next generation and the other test litters were discarded. One male from each of the ten sibships was used in the test matings, and the females were arranged in harems of three or four per male. The females of a harem all came from different sibships, so that each male was mated to a set of females unrelated to each other and to him. Any difference between the mean litter sizes of harems, in excess of what would be expected from sampling, would therefore be attributable to differences of male fertility. Only the first 16 generations have so far been analyzed in this way. The analysis of variance (table 4) refers to the variation within generations, pooled over the generations. Because of the non-random, but also irregular, distribution of the sibships among the harems, this analysis is an awkward one and the compositions of the mean squares have not yet been worked out. The best thing to do meantime seems to be to remove the variation attributable to sibships and use the residual mean square as the error variance. This makes the mean square between harems significant at the 1% level and, though the significance is certainly overestimated, there seems to be little room for doubt that males do influ-

ence the size of the litter they sire. The component of variance attributable to males works out to be about 10% of the total variance. This, however, is an upper limit because the mean square for harems contains some variation attributable to sibships which has not been recognized in the analysis. Whatever the precise figure may be, we can conclude that the influence of the male parent in determining litter size is small compared with that of the female parent and the litter itself.

Parent-offspring correlation. The control line provides data for the estimation of the parent-offspring correlation, though the data have not yet been fully analyzed in this respect. The data, of course, accumulated as the experiment went on and the information was not available for the prediction of the expected response to selection. If it had been, the selection would probably not have been attempted because the correlation is virtually zero. An analysis of the first 16 generations yielded a daughter-dam regression of -0.066 ± 0.053. A graphical representtation of the relationship between daughters' and mothers' litter sizes based on the whole experiment shows clearly the absence of any correlation (see figure 5).

The correlation between mothers' and daughters' litter sizes is, however, complicated by an interesting maternal effect. The litters in this experiment were not adjusted to a standard size at birth. Under this system mothers who have large litters rear their daughters in a large litter. The daughters are consequently retarded in growth and this tends to make them have small litters. In this way the maternal effect contributes negatively to the correlation between mothers and daughters, and the correlation observed is th combination of this negative environmental correlation balanced against an positive genetic correlation that there ma be. The two component parts of the cor relation can be separated to some exten by taking account of the daughters' bod weights, which provide a measure of a least part of the maternal effect. If weigh is held constant, then the (partial) regres sion of daughters' on dams' litter size be comes $+0.058 \pm 0.053$. Doubling the re gression gives an estimate of 11.6% fo the heritability of litter size, but the larg standard error renders any value betwee 0 and about 30% compatible. Apart fron the distressing magnitude of its standar error, this is not a completely satisfactor estimate of the heritability because th standardization of body size eliminate not only the unwanted maternal effec but also the variation of litter size that i associated with genetic differences of bod size. The heritability will therefore b underestimated, and the responses to se lection do indeed indicate a higher value

SELECTION

Selection for increased litter size wa made in one line, referred to as the "high line," and for decreased litter size in an other line, referred to as the "low line." The selection in both lines was carried ou in the following way. As in the contro line, each generation consisted of ten full sib families. All the females in each fam ily were test-mated but, unlike the contro line, sisters were mated to the same male When the test litters had been born, selec tion was made within the sibships. Tha is to say, in each sibship the female with the best litter was selected. Her litter was

TABLE 4

Analysis of variance of litter size within generations of the control line up to generation 16

The F ratios, both significant at the 1% level, and variance components are only approximate for reasons explained in the text.

Source of variation	d.f.	M.S.	F	Variance component	
					%
Harems	138	4.49	1.47	0.45	10.6
Sibships	139	5.41	1.77	0.73	17.3
Residual	204	3.06		3.06	72.1
Total				4.24	100.0

Mean number of litters per harem, 3.48
Mean number of litters per sibship, 3.46

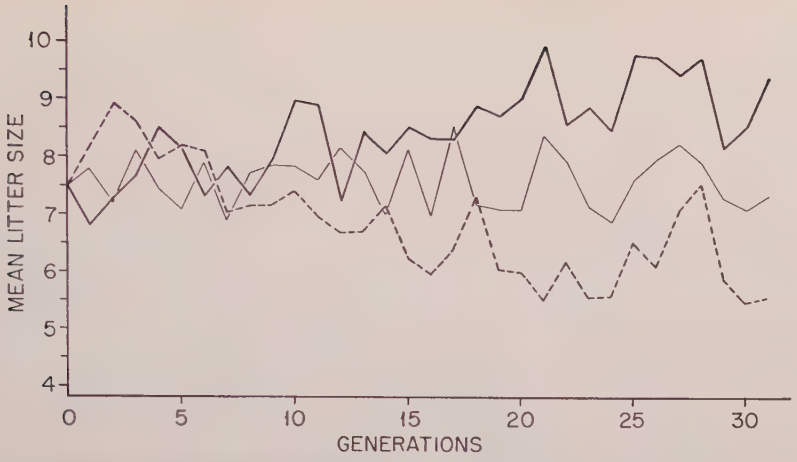

Fig. 2. Response of litter size to selection. Heavy line, selection for large litters; light line, unselected control; broken line, selection for small litters.

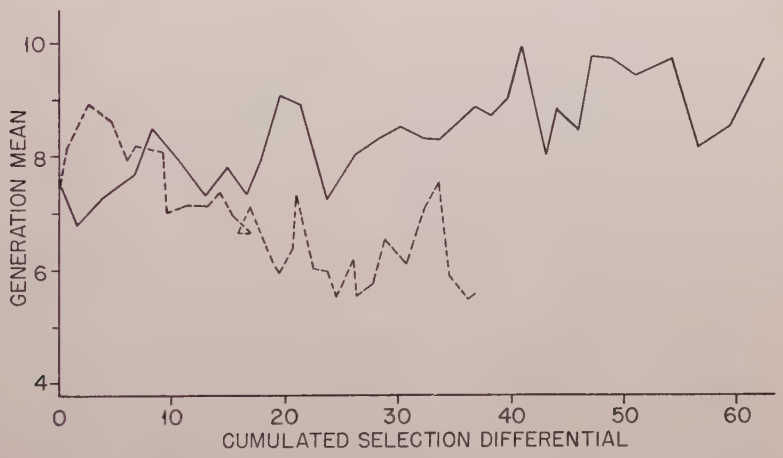

Fig. 3 Response of litter size to upward and downward selection.

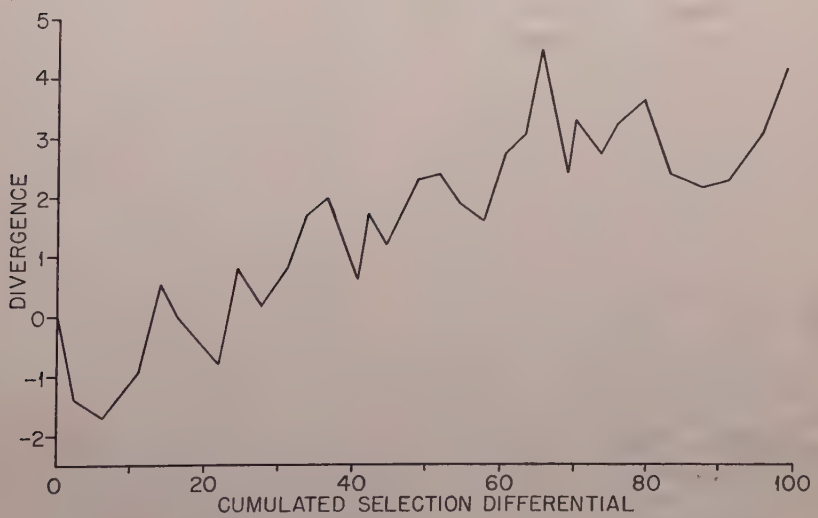

Fig. 4 Divergence between upward and downward selected lines.

reared for testing in the next generation and the other test litters were discarded. This procedure amounted to within-family selection applied to females, males being taken at random. Because the selection was made within families the negative maternal effect was circumvented, each group of females among which selection was made having been subjected to the same maternal environment.

Response to selection. The responses to selection are shown in figure 2 plotted against the generation number, and in figures 3 and 4 plotted against the cumulated selection differential. The results are fairly straightforward and need not be discussed in detail. There was a contrary response in the first two generations, attributable to the negative maternal effect mentioned earlier. Thereafter, the responses went in the right directions and continued till about generation 20 when both lines ceased to respond. The rates of response shown by figures 3 and 4 must be doubled to give the realized heritabilities because only one sex was selected. The realized heritabilities, estimated roughly from the graphs and discounting the contrary response in the first two generations, are 8.3% for upward selection, 22.9% for downward selection, and 12.6% for the divergence between the high and low lines. Thus there was a marked asymmetry in the responses, downward selection responding at nearly three times the rate of upward selection. I do not know the reason for this. These realized heritabilities refer, of course, to within-family selection, and are not directly comparable with the estimate from the daughter-dam regression. Conversion to individual heritabilities gives values of 14.5% from the upward selection, 40% from the downward selection, and 22% from the divergence. These values are considerably higher than the value of 11.6% obtained from the daughter-dam regression. But the daughter-dam regression, as noted before, referred to variation in litter size not associated with genetic variation in body size, whereas the selection could make use of any such variation. I therefore do not regard this discrepancy as a serious one.

The final levels reached, after the r sponses had ceased, were 9.2 young i the high line, and 6.0 in the low, com pared with a mean of 7.6 in the contro Thus an improvement of about 1.5 youn per litter was made in both direction and the final difference between the hig and low lines was 3.2 young. This di ference amounts to 1.6 times the origina phenotypic standard deviation and 3. times the additive genetic standard devia tion. This total response to selection i very small compared with responses i other experiments, which commonly yiel some 10–20 phenotypic standard devia tions of response, or 20–30 additive gen etic standard deviations. The conclusio to be drawn may be either that relativel few genes are concerned with the varia tion of litter size, or the limits to selectio do not represent fixation at all relevan loci. The latter seems the more probabl because lethal and semilethal genes which may cause variation of litter size through their effects on embryonic viability could not be brought to fixation.

Nature of the changes made by selection. One circumstance has so far been omitted from the consideration of the responses to selection, and that is the fact that the females of the high line were reared in large litters and the females of the low line in small litters. Should not some adjustment be made for the differential maternal effect so produced? In order to explore this problem I calculated over generations 20-31 in the high and low lines, the mean daughters' litter size for each maternal litter size, hoping thereby to make a comparison between the lines at a standard parental litter size. The resulting regressions of daughters' on dams' litter sizes are depicted in figure 5 along with a similar calculation for the control line over the whole experiment. The situation revealed is very striking. The control line, as mentioned earlier, shows no correlation at all between daughters and dams, but the high line shows a negative correlation and the low line a positive one. It is not clear to me how this information should be used to make a just comparison between the lines because, in the first place, the genetic properties of the three lines are clearly different, and in the sec-

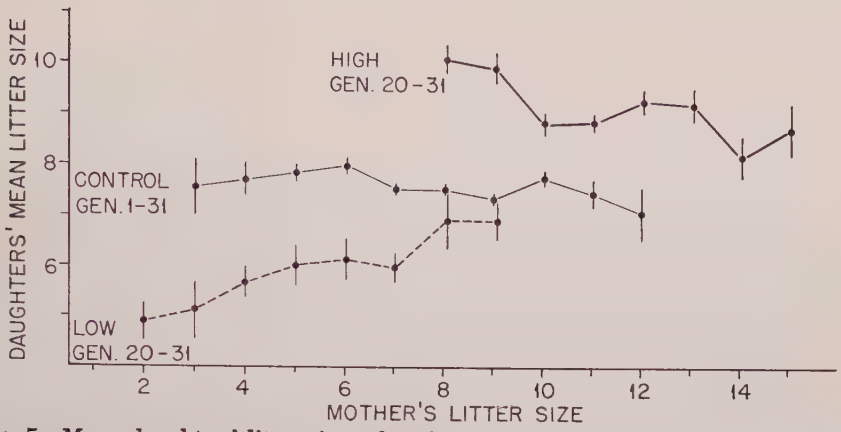

Fig. 5 Mean daughters' litter sizes plotted against the mother's litter size, showing the regression of daughters' on dam's litter sizes. Vertical lines extend to ± one standard error.

ond place, the difference between each of the selected lines and the control will vary according to the parental litter size taken as standard. Perhaps they should be compared at a standard parental litter size of 7.5, which is the initial level. Then the high line is about 2.6 young above the control and the low line about 1.0 below. The asymmetry of the unadjusted responses might be accounted for by invoking the maternal effect in this way, but I do not feel confident enough to pursue the matter here.

The different genetic properties of the selected lines brought to light by the daughter-dam regressions depicted in figure 5 lead to another line of thought, which, in conjunction with the facts to be mentioned later, leads to a hypothesis about the nature of the genes that have been responsible for the responses to selection. A plausible interpretation of the difference between the daughter-dam regressions is that selection for increased litter size had exhausted the additive genetic variance, so that what is left at the end is the negative maternal effect; whereas selection for reduced litter size had increased the genetic variance, so that the genetic correlation overweighs the environmental and the daughter-dam regression becomes positive. An increase of genetic variance in the low line would be compatible with the hypothesis that low litter size is due to lethal and semilethal genes in the embryos. These genes, presumably at low frequencies initially, could be brought by selection to intermediate frequencies, but not beyond; and at intermediate frequencies they would make their maximum contribution to the variance of litter size. In this way the response to downward selection would cease when the daughter-dam regression was at its maximum.

This idea of lethal genes in the low line was suggested by the distributions of litter size which differ strikingly between the lines. These distributions are shown in figure 6A, where the high and low line distributions refer to generations 20–31 and the control line distribution to the whole experiment. The distributions of the high line and control have a small "tail" at low litter sizes, whereas the distribution in the low line looks as if it were a compound of two distributions—one with a mean at the mean of the control and the other with a mean corresponding to the tail of the control distribution. If we suppose that the tail represents litters that are segregating a lethal, then an increase of the frequency of these litters in the low line would satisfactorily account for the altered form of the distribution in the later generations of the low line.

The final investigation to be described here concerns the ovulation rate in the selected lines. The ovulation rates were determined by egg counts after natural mating. Two sets of counts were made, one at generations 16 and 17, when only the selected lines were counted, and the other at generation 31, when all three lines

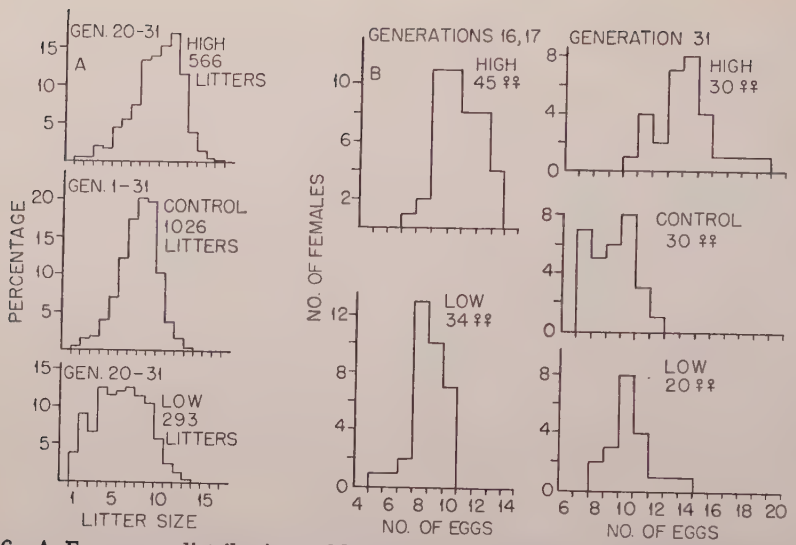

Fig. 6 A. Frequency distributions of litter size. B. Frequency distributions of numbers of eggs ovulated in natural matings.

were counted. The results are given in table 5, and the distributions of the egg numbers are shown in figure 6B. It is immediately clear that selection, unlike inbreeding, has changed the ovulation rate. If the ovulation rates are compared with the litter sizes in the high and low lines, as shown in table 5, then it appears at first sight that the difference in ovulation rate alone is enough to account for the difference in litter size. But if we deduce the loss of eggs and embryos from the difference between the ovulation rate and the corresponding litter size, we find that this conclusion is not fully justified. The losses must be compared on a proportionate, or percentage, basis, and then the loss is greater in the low line than in the high line. This fits in well with the lethal hypothesis. The ovulation rate in the low line at the end of the experiment is no lower than that of the control, and thi also agrees with the lethal hypothesis, be cause the distributions of litter size sug gest that the low line has a mode equal t the mode of the control line, which sug gests in turn that the size of undeplete litters is the same in the two lines. I should be mentioned, however, that th ovulation rate in the low line is in fac significantly higher than that of the con trol; the reason for this is not clear.

My tentative conclusion about the na ture of the changes produced by selectio is this: that selection for increased litte size has acted chiefly on the fertility o the females by increasing ovulation rate though there has at the same time beer an increase in the proportion of eggs o embryos lost; selection for decreased litte

TABLE 5

Ovulation rates and deduced losses

	Generations 16 and 17		Generation 31		
	High line	Low line	High line	Low line	Control
Number of eggs[a]	10.4	8.5	13.7	10.3	8.9
Difference (high–low)	1.9		3.4		
Litter size	8.4	6.2	9.2	6.0	7.6
Difference (high–low)	2.2		3.2		
Number lost	2.0	2.3	4.5	4.3	1.3
Percentage loss	19	24	33	42	15

[a] All differences significant.

ze, in contrast, has acted chiefly on the ability of the embryos, and has resulted little or no decrease of ovulation rate it a marked increase of embryonic mortality.

DISCUSSION

We have tried, in the investigations reewed here, to break down the character itter size" into its component characters, id have found that these components ive different genetic properties. In parcular, ovulation rate is influenced by nes with predominantly additive effects i it (more precisely, without directional minance), whereas implantation rate nd embryonic viability are probably influnced more by deleterious recessive alleles low frequencies. To understand the asons for these differences, we shall ave to discover the relationships of litter ze and its components with natural fitess. About the nature of these relationips, however, I can at present offer little ore than conjecture.

On the analogy of clutch size in birds, ne would expect an intermediate litter ze to confer maximal fitness—that is, to ield the greatest number of adult offpring—and the existence of a fair amount f additive genetic variance of litter size in accord with this expectation. I have, owever, been unable to find any evidence f an intermediate optimum in laboratory ice: the number weaned begins to drop ff only when the litter size exceeds about 3 born alive, and there are very few eaths after weaning. The situation in ne wild, with a limited food supply, is robably very different, and the genetic roperties of litter size in laboratory mice ay perhaps reflect an adaptation to conitions in the wild. But it is difficult, evertheless, to believe that this adaptaon could survive so long under domestiation when the pressure of natural selecon was shifted toward higher litter sizes.

If we accept the postulate that an interediate litter size is optimal, or has been the past, then the different genetic propties of the component characters are eadily understandable. It is inconceivble that the implantation rate or emryonic viability should have intermediate ptima; any loss is wasteful of maternal ffort and the upper extreme values must represent maximal fitness. Depression on inbreeding is just what would be expected of such characters and what was in fact found. Adjustment of the litter size to an intermediate optimal value would then be achieved best by an appropriate ovulation rate. It is therefore in the ovulation rate that we should expect to find the genetic properties of a character with an intermediate optimum. Such a character should respond to selection and not change on inbreeding, and this is what was found.

SUMMARY

Litter size, measured as the number of live young born in first litters, was studied as a quantitative character by inbreeding and by selection; the changes produced were investigated by dissections of pregnant females.

Inbreeding led to a decline of about 0.5 young per 10% increase of the inbreeding coefficient, and the decline was linear with respect to the inbreeding coefficient. Three out of 20 lines survived to about 90% inbreeding and one of these survived indefinitely. These lines reached high levels of inbreeding without any decline of litter size. This suggests that overdominance cannot have been a major cause of the inbreeding depression. About 40% of the reduction of litter size on inbreeding was attributable to reduced fertility of the females and the remaining 60% to reduced viability of the young. The reduced fertility of inbred females was due almost entirely to an increased pre-implantation loss of eggs or embryos. The ovulation rate was not influenced by inbreeding.

An unselected control line, maintained with minimal inbreeding, did not decline in litter size though by the end (generation 31) its computed inbreeding coefficient was 32%. Environmental differences between generations contributed only about 2½% of the total variation of litter size.

Differences of fertility between males in the control line contributed, at the most, 10% of the variation of litter size. The correlation between parents and offspring was virtually zero, but this is complicated by a maternal effect that contributes negatively to the correlation and counterbalances the positive genetic component of the correlation.

Selection applied to females within families yielded progress with a realized heritability of 8% for upward selection and 23% for downward selection. Progress ceased after about 20 generations of selection, when the mean litter sizes were 9.2 young in the high line, 6.0 in the low, and 7.6 in the control. Selection, unlike inbreeding, affected the ovulation rates, the mean ovulation rate in the high line being 3.4 eggs greater than that of the low line. But the low line females ovulated more, not fewer, eggs than the control.

Comparisons of the properties of the lines after the response to selection had ceased suggested the tentative hypothesis that, whereas the response to upward selection had been achieved through an increased ovulation rate, the response to downward selection resulted from a reduced viability of the embryos.

OPEN DISCUSSION

RUNNER[1]: May I introduce some points about litter size that a genetic analysis may overlook? It was heartening to hear Dr. Falconer include genetic effects acting through maternal physiology as a possible mechanism by which size of litters may be altered.

Size of litters as expressed in the current presentation was based on those females that succeeded in becoming pregnant. Persons who experiment with inbred mice customarily find that about half the females that have shown a vaginal plug fail to show signs of pregnancy, i.e., implantation sites. I believe that these failures are part of the fertility picture, for almost all such females initially had fertilized eggs. As part of the picture, such females should be reckoned having lost the entire litter, i.e., litter size of zero, when making an assessment of reproduction.

A second point for consideration may be introduced by the question of why do females with fertilized eggs fail to become pregnant. This involves qualitative consideration of the corpora lutea counted by Dr. Falconer. To me it is a comparatively safe generalization that almost 100% of mated inbred female mice possess fertilized eggs and develop corpora lutea. These corpora lutea, however, do not invariably maintain pregnancy; indeed they do not always s port pseudopregnancy. There exists transition between those clutches of pora lutea that fail to support pregna and those that are associated with a quota of live births. In other words, p nancy in a polytocous female is not an or-none phenomenon. This means that the intermediate range, females are ba able to support implantation and maint pregnancy and, although the corpora lu counts may be high, a relatively small p portion of the potential number of yo may be assessed at birth. In other wo quantitatively the corpora lutea may i cate a large initial litter size but qual tively one or all of the corpora lutea n be substandard and cause either pre- postimplantation death of otherwise via embryos.

A third point about genetic conside tions of maternal factors regulating size litter is the surprising sensitivity of potentially high quality corpora lutea environmental factors. This has been de onstrated in our laboratory by interrupti pregnancy, litter size of zero, by handli the mother, and in a companion exp ment by maintaining these pregnanc and protecting the embryos with prog terone. It has further been shown t mated females destined to have quali tively faulty corpora lutea can be gi progesterone, thereby enabling more e bryos to survive, with a resultant incre in litter size. The sensitive interacti between maternal physiology (genetic c stitution) and subtle environmental in ences have frequently been shown to important in litter size.

The importance of maternal genetics litter size suggests a need to distingui between prenatal loss resulting from e bryonic lethal factors and from mater factors. Considerations of such items litter size of zero, qualitative nature corpora lutea, and subtle environmen factors on litter size would caution agai a temptation to interpret prenatal l synonymously with genetic lethals.

FALCONER: I fully agree that mater factors, such as progesterone deficien are possible causes of the prenatal loss the low line, and I must repeat that

[1] M. N. Runner, National Science Foundati

pothesis that embryonic lethals are re-
nsible is based on no direct evidence.
may well prove to be wrong. With re-
d to Dr. Runner's first point: I do not
nk that the exclusion of zero litters
m the estimation of the mean litter sizes
kes much difference in this case be-
se their numbers were rather small.
e numbers of females that produced no
er or no live young in their litter were
in the control, 4% in the high line,
d 7% in the low line, averaged over the
t seven generations.

BATEMAN[2]: I was surprised, Dr. Fal-
ner, that you are equating the maximum
mber weaned to the optimum litter size,
cause in view of what we know about
e effect of litter size on fertility of the
ogeny, it is obvious the fertility of the
aned is going to be a determining fac-
. That is going to pull your optimum
wn very likely to somewhere around
ght.

FALCONER: I agree that in assessing
e optimal litter size one must take ac-
unt not only of the number of children
oduced but also of the number of grand-
ildren: large litters mean more children
t perhaps fewer grandchildren. But I do
t think that the fertility of the daughters
ll make much difference to the optimal
ter size, because a larger number of
ughters will compensate for a consider-
le reduction of their fertility.

BATEMAN: One other point. It surprised
e very much that selection did not pull
wn the litter size below six. One would
ink it would be the easiest thing in the
orld to reduce litter size practically to
ro.

FALCONER: The chief obstacle to re-
cing litter size to practically zero is the
minishing amount of selection that can
applied: the smaller the selected litters,
e fewer the daughters among which to
lect in the next generation.

BRADEN[3]: In your selection for litter
ze, I understand that you mated the mice
a standard age of 6 weeks or there-
out. This would mean that mice from
rge litters would weigh less than those
om small litters. Do you expect that
ating the mice at a standard weight
ther than age would have prevented the
rly effect you noted of the response be-
ing in the opposite direction to the selection?

FALCONER: Yes, I think it would. A regression analysis proved that daughters' and mothers' litter sizes are positively correlated if the daughter's weight at 6 weeks is held constant.

E. S. RUSSELL[4]: I think the factors responsible for fertility must be quite different in some cases, at least, between different inbred populations. This is based on a practical experience we have had recently at the Jackson Laboratory. I think most of you recognize that C3H is a fertile inbred strain and that DBA/2 is a fertile inbred strain. Both of these have behaved quite well. Mating a C3H female with a DBA/2 male gives, as we usually expect, great hybrid fertility. However, mating a DBA/2 female with a C3H male gives nothing at all. So the factors responsible for the fertility of C3H must be rather different from those that are responsible for the fertility of DBA/2, i.e., qualitatively different.

LUSH[5]: With reference to an intermediate weaning number being optimum, I would mention a study of swine (Dickerson *et al.*, '54) in which we tried to figure out how much of the selection was automatic and how much was extra. The automatic would result from such facts as that, in a litter of eight, there would be twice as many chances to pick one for breeding as from a litter of four. Roughly four-fifths of all the selection we were able to practice was automatic. We thought we had been selecting heavily for litter size, but the extra pressure we were able to put on that was comparatively slight.

That raises the question that, if nature has been doing such intense selection over many generations, why is litter size staying put. Perhaps studying the grandchildren might give us a key to it. We did not try to measure selection intensity by counting the grandchildren.

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[2] A. J. Bateman, Christie Hospital, Manchester.
[3] A. W. H. Braden, Commonwealth Scientific and Industrial Research, Prospect, New South Wales.
[4] E. S. Russell, Roscoe B. Jackson Memorial Laboratory.
[5] J. L. Lush, Iowa State University

WRIGHT[6]: One of the striking results in the early study of 23 inbred lines of guinea pigs was a rather strong correlation between the mean size of litters of the lines and their mean weights corrected for size of litter. I think that this holds in a good many other cases. Thus, in MacArthur's selection of strains of mice for large and small size, there seemed to have been automatic selection for large and small litters, respectively. Gregory found a similar correlation in strains of rabbits. I have always thought of this as probably relating to the number of corpora lutea. Heredity for large size may carry with it production of a large number of ova.

FALCONER: There is certainly a genetic correlation between body size and litter size in mice also. We have found that litter size has always changed in response to selection for body size, and I think that body size has changed in response to selection for litter size, though I cannot yet be certain of this. We found a positive correlation between the number of corpora lutea and the weight of the female, so at least part of the effect of body size on fertility operates through the ovulation rate.

BRADEN: Litter size is inversely correlated with the body weight of the baby mouse. But is this maternal effect on weight also in evidence in the mature body weights?

FALCONER: I think it probably is. Brumby ('60) showed that maternal effects on weight persisted at least to 12 weeks of age.

BRADEN: In inbred strains we found that there was virtually no correlation between the size of the litter in which the animal was born and ovulation rate of the animal when mature.

RODERICK[7]: You have shown that the high and low lines differed in the magnitude of their response to the selected character. Do you have any data on the comparison of the selection differentials?

FALCONER: Yes. The selection differential in the high line was about two mice per litter, and in the low line about one.

RODERICK: Did you synchronize the generations of the lines? And did you find that the low line held you up?

FALCONER: Yes, the generations were synchronized throughout. The low line did not hold us up; if anything, it was control that tended to be slowest in bre ing.

AUSTIN[8]: The large preimplantat losses are reminiscent of those that see after artifically induced ovulation rats and mice. These, we found, were tributable to a number of factors, am which failure of fertilization was of c paratively minor importance. A num of eggs failed to cleave normally, and put this down to the fact that they m have been bad eggs. But also a num of apparently normal cleaving eggs fai to implant, and we attributed this to same cause that Dr. Runner has name faulty corpora lutea function. Have tested this possibility by injecting prog terone?

FALCONER: No, but I certainly th that should be tried.

LUSH: Some of these things are clutt ing the classroom lectures or textbook lustrations of pleiotropy. That is, wher gene has one effect on maternal perfor ance and another effect in the offspri this makes pleiotropy operationally p sible but difficult to test or measure. T possible genetic control of corpora lut functioning might be just such a case. you get the impression that it is rare very common for a gene to have one eff in an individual and a different effect its parents as, for example, for litter s to affect the size of the young and (throu that) to have a different effect on size litters the young produce? Do you thi that sort of thing is exceptional, or do y think you see it in nearly all character

FALCONER: I think that may well be common situation, particularly in ma mals where maternal effects are so i portant.

LUSH: Both a maternal effect and th an individual effect, possibly different sign or kind?

FALCONER: Yes.

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[6] S. Wright, University of Wisconsin.
[7] T. H. Roderick, Roscoe B. Jackson Memor Laboratory.
[8] C. R. Austin, National Institute for Medi Research, London.

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ɔenetic Analysis of Induced Deletions and of ɔontaneous Nondisjunction Involving ɔromosome 2 of the Mouse

LIANE BRAUCH RUSSELL AND W. L. RUSSELL
Biology Division, Oak Ridge National Laboratory,[1]
Oak Ridge Tennessee

The specific locus method, which is ng used in comparative mutation rate periments (e.g., Russell, '51; Russell, ssell, and Oakberg, '58; Russell, Rus- l, and Kelly, '58), has resulted in the cumulation of large numbers of inde- ndent mutations, which are potential ɔls for detailed genetic analyses of spe- ic regions of chromosomes. Such anal- es have already revealed basic phenom- a that are new for mammalian genetics. ɔme of these, e.g., the existence of riegated-type position effects from X- itosome translocations, have been re- ɔrted elsewhere (Russell and Bangham, 9, '60).[2] The present paper will discuss veral other basic findings that have come light in the course of the exploration of region marked for the detection of point utations versus deletions, namely, the *lute-short-ear* region. For earlier results ncerning this region, reference may be ade to some of our past publications ited in connection with specific points).

The specific locus method consists in ating irradiated or control wild-type ani- als to a test stock homozygous for a ɯmber of markers (*a/a b/b* $c^{ch}p/c^{ch}p$ *se/d se s/s* in our experiments) so that utants can be detected in F_1. Among e seven loci used in our specific locus utation-rate studies two—*dilute, d,* and *ort-ear, se*—were chosen because of eir extremely close linkage (crossover equency = 0.16%). It was hoped at the tset that they would provide some an- vers concerning the relative frequencies deletions and point mutations among duced and spontaneous genetic changes. ıe possible mutants involving these two ɔi are *'d'* + */d se*, + *'se'/d se*, and *'d se'/d* . (Throughout this paper newly arisen mutant genes will be designated by enclosure in single quotation marks to distinguish them from the test-stock markers.)

As soon as the data for irradiation of spermatogonial stages began to accumulate, it became apparent that, although there was a considerable frequency of mutations at the *d* locus and some mutations at the *se* locus, animals mutant for *both* dilute and short-ear did not occur (Russell, '51; Russell and Russell, '59). In the beginning, an explanation for this lack that had to be considered was the possibility that *d* and *se* were actually separated by a considerably greater distance than was indicated by their crossover frequency and that, consequently, deletions involving both of them were long enough to act as dominant lethals. However, as will be shown, animals mutant for both *d* and *se* were obtained shortly afterward in experiments with postspermatogonial stages (Russell *et al.*, '57; Russell, Bangham, and Gower, '58) and oocytes (Russell, Russell, Gower, and Maddux, '58; Russell, Russell, and Cupp, '59). It, therefore, became clear that *d* and *se* could be deleted together without giving a dominant lethal effect.

There remained two alternative explanations of why *d se* mutants were not obtained from irradiation of spermatogonial stages: either they were not induced by radiation at that cell stage; or, they were induced but not transmitted, there being, possibly, some stage after the reduction division of meiosis when the deletion-bearing cells were either inviable or at a functional disadvantage (this

[1] Operated by Union Carbide Corporation for U. S. Atomic Energy Commission.

[2] This subject was included in the oral presentation at Gatlinburg.

might be referred to, briefly, as a postreductional hurdle). The fact that some spontaneous occurrences of presumed *d se* mutations seemed to be not transmitted appeared to support the alternative of a postreductional hurdle, and it was expected that *d se* deletions would be obtained from irradiated spermatozoa and possibly other postspermatogonial stages. This was, indeed, the case, but new problems arose when these turned out to be transmitted (Russell *et al.*, '57; Russell and Russell, '59), favoring, again, the idea that *d se* deletions were actually not induced in spermatogonia (Russell, Russell, and Oakberg, '58).

Further work with some of the previously reported and more recently obtained *d se* mutants has helped to resolve some of these problems and has yielded additional genetic information on the *d se* region. Several topics will be discussed in the present paper. The problem of degree of transmission will be taken up first. A second question to be considered is whether there are mechanisms, other than deletion, by which presumed *d se* mutants can be obtained. It will be shown that the available evidence indicates that presumed *d se* mutants are of at least two types. Third, the correlation between cell stage and type of genetic change involving *d* and *se* will be discussed. Finally, some evidence concerning the finer structure of the *d se* region will be presented.

I. TRANSMISSION

A. *Criteria for disturbance in transmission.* The general consequences of assuming a "postreductional hurdle" (see Introduction) to transmission of a given mutation (the term is used here broadly for any genetic change at all) may first be considered in terms of germ cell stage in which the mutation arises. If a mutant animal is the result of a genetic change that has occurred in *gonial* cells of the parent, the very existence of the mutant proves that the mutation must have passed any postreductional hurdle; and it is, therefore, expected that the mutant animal will transmit the mutation, at least to some degree. This is similar for mutants that are the result of mutations that have occurred in primary oocytes or primary spermatocytes. On the other hand, it not necessary to assume that a mut animal that is the result of a mutation a spermatozoon will transmit, since mutation has perhaps not yet had to p the presumed hurdle. This is also true mutations arising in spermatids, provid one limits the assumptions as to just wh the hurdle occurs.

Our specific-locus experiments ha yielded 15 separate occurrences of p sumed *'d se'/d se* mutants. Their orig was as follows: the groups of anim derived from irradiated postspermato nial stages, oocytes, and spermatogon contained 6, 4, and 1, respectively; and occurred in controls. It should be reme bered that mutations found in anima derived from irradiated germ cells ma of course, be of spontaneous origin. As matter of fact, the control frequency such that the single *d se* mutant in t group derived from irradiated spermat gonia could have been of this type, could a small proportion of the oth mutants.

According to the reasoning outline some or all of at least 10 "mutation might, when tested, show nontransmi sion or low transmission. These wou include the six derived from irradiate postspermatogonial stages and the fou from controls (where cell stage of origi is unknown). In addition, there is, course, the possibility of finding nontran mitters among the other *d se* mutants, one allows for the chance that they coul have been of spontaneous origin.

The criteria by which disturbances o transmission are recognizable are outline in table 1. Complete failure of transmis sion, resulting from what may loosely b called gamete lethality (although mecha nisms more complicated than outrigh lethality could be involved), would o course result in the mutant yielding n *'d se'*-bearing progeny. If the deficien chromosome is not completely "gamet lethal" but results in the failure of onl some of the gametes, then the proportion of *'d se'*-bearing progeny of the mutan will be reduced to less than the normally expected 50%. The same result, however would be obtained for a *completely* trans mitted *'d se'* with a dominant subvital ef-

TABLE 1

Criteria for distinguishing between various possible actions of 'd se'

Hypothesis for action of *'d se'*	Expected consequences			
	Proportion of offspring carrying *'d se'*		Proportion of normal litter size	
	From ♂ parent	From ♀ parent	From ♂ parent	From ♀ parent
Dominant subvital	< 0.5	< 0.5	$< 1, > 0.5$	$< 1, > 0.5$
Gamete semilethal	< 0.5	< 0.5	1	$< 1, > 0.5$
Gamete lethal	0	0	1	0.5

ect. A distinction between these alternatives can be made on the basis of the itter sizes of transmitting animals. In he case of a dominant subvital effect, the itter size of both male and female parents hould be reduced, since the upset ratios re due to death of *'d se'*-bearing progeny *n utero*. On the other hand, if the *gametes* of the transmitting animal are in ome way affected by *'d se'*, then it is quite ossible that litter size of a poorly transnitting male would not be affected, since he number of functional sperm remaining could be sufficient to fertilize all the ggs of his mate. Litter size of a poorly ransmitting female would, of course, be epressed since the adversely affected ggs could not be replaced in a given vulation. To summarize: if it should be ound that heterozygous males produced ormal litter size, in spite of reduction in he proportion of their mutant progeny, his would be indicative of reduced transnission—as opposed to inviability of hetrozygotes.

B. *Progeny of the 'd se'/d se mutants.* These two parameters—percentage of *d se'*-bearing progeny and litter size—are isted in table 2 for the original mutant nimals themselves. The results for male nd female mutants are listed in separate olumns. As will be shown, percentage f *'d se'* bearers in the progeny of the orignal mutants could not be determined on he basis of phenotype and is, therefore, ased only on those offspring which were ndividually tested. Average litter size is ased on somewhat larger numbers.

Of the 15 separate occurrences of *d se*, here are two for which the data are indequate to settle the question of transnission: in one case (200G), the mutant vas sterile; and in the other ($23Z_b$), the nutant died before a sufficiently large number of progeny could be obtained to give conclusive results. The remaining 13 have been classified into four groups according to the proportion of their progeny bearing the *'d se'* chromosome (see extreme right column): (1) mutants producing approximately the expected 50% *'d se'* (designated as giving +++ transmission); (2) mutants producing only about two-thirds of the expected proportion (++ transmission); (3) mutants producing very few *'d se'* progeny (+ transmission); and (4) mutants producing presumably no *'d se'* progeny ("zero-transmitting" mutants). Consideration of this last category will be reserved for section II. For the first three categories, it will be noted that, while the litter size of female transmitters goes down as percentage *'d se'* goes down, the litter size of male transmitters remains approximately normal. This finding suggests the possibility that the mutant *'d se'* in some way affects the gametes, as discussed. Some further evidence bearing on this point will be considered after the mating schemes used have been described.

Throughout the remainder of section I, transmitted *'d se'* 's are referred to as deletions. Although presumed *'d se'/d se* mutants could arise through a number of mechanisms, it will be shown in section II that deletion seems the most likely one for those mutants which transmit *'d se'*.

C. *Mating schemes.* In specific locus experiments, the mutations are recovered in combination with the test-stock recessives. This leads to some difficulties in the determination of the relative proportions of the mutant *'d se'* chromosome (designated throughout by single quotes) and the test-stock *d se* chromosome among the progeny of a *'d se'/d se* mutant. The method we have used is based on our finding that *'d se'* deletions in combination

TABLE 2

Origin, size, and progeny of the original d se mutants

Mutant symbol	Size of mutant animal	Origin of mutation (stage irrad.)	Progeny of mutants						Transmission[d]
			Male mutants			Female mutants			
			Mean litter size[a]	No. F_1 classified	% 'd se'	Mean litter size[a]	No. F_1 classified	% 'd se'	
43 CoS	OK	Spermatogonia	—	—	—	6.7(10)	62	61.3	+++
17 Z_b	Small	Oocytes	7.9(9)	67	43.3	—	—	—	+++
130 G	OK	Spermatozoa or spermatids	—	—	—	7 (1)	6	(50.0)	+++?
99 G	Small	Spermatozoa	—	—	—	5.1(9)	34	35.3	++
209 G	Small	Spermatozoa or spermatids	6.0(8)	59	37.3	—	—	—	++
8 Z_b	Small	Oocytes	6.0(6)	31	12.9	—	—	—	+
2 CoS	Small	Spermatozoa	—	—	—	2.9(7)	19	10.5[c]	+
201 G	Small	Spermatozoa	—	—	—	2.8(8)	21	9.5	+
28 FRO_d	OK?	Control	6.4(20)	70 52[b]	0	—	—	—	0
204 K	OK?	Control	8.3(12)	30	0	—	—	—	0
60 PB	OK	Control	—	—	—	5.7(7)	20	0	0
39FUTh	OK	Oocytes	—	—	—	7.8(12)	70 39[b]	0	0
17 BS	Small	Control	—	—	—	6.0(8)	26	0	0
200 G	Small	Spermatozoa	—	Sterile	—	—	—	—	?
23 Z_b	OK?	Oocytes	8 (1)	6 6[b]	0	—	—	—	?

[a] The figure in parentheses is the number of litters on which the mean is based. Litters born to females more than 1 year old are excluded from calculations of mean litter size.

[b] Represents number of F_1 double tested (see table 6).

[c] This value may be an underestimate. It is based on two ++/*'d se'*, both females, among the 19 tested F_1. However, an additional two F_1 males were sterile. If these were indeed ++/*'d se'*, percentage *'d se'* would be 19.0. Sterility in F_1 has not complicated the testing of other mutants.

[d] See text, section IB.

with d^1 produce opisthotonic progeny. The gene d^1, which has been described by Searle ('51, '52) and to which several independent mutations have occurred (e.g., Russell, '51; Russell and Russell, '59; also, see section IV), is an allele, or possibly pseudoallele, of maltese dilution, *d*. The combinations d^1/d^1 and d^1/d are indistinguishable from d/d in coat color, but d^1/d^1 animals develop convulsions with opisthotonus and die; d^1/d animals are normal. It was found that *'d se'*$/d^1+$ was indistinguishable from d^1/d^1, i.e., dilute and opisthotonic; *d se*$/d^1+$ is, of course, dilute and neurologically normal.

The *'d se'*/*d se* mutant animal was mated to wild type (fig. 1, line 2) and large numbers of F_1 animals obtained. Each of these F_1's was individually tested with a mate heterozygous for d^1 (fig. 1, line 3). Those F_1's which produce opisthotonic progeny carry the *'d se'* deletion, and the F_1's producing dilute, neurologically normal, progeny carry the test-stock *d se* markers (fig. 1, line 6). It should be pointed out that the method does not rely on the *absence* of a type; on the contrary, no animal was considered tested until it had produced some dilute progeny, either neurologically normal or opisthotonic. Furthermore, this method has two great advantages over the alternative one of mating the original *'d se'*/*d se* mutant itself to d^1 heterozygotes. First, the proportion of *'d se'* transmitted is based on ++/*'d se'* versus ++/*d se* progeny, i.e., with *'d se'* in its presumably most viable combination; rather than on d^1+/*'d se'* versus d^1+/*d se* progeny, which may yield unreliable ratios owing to the inviability of the opisthotonic type. Secondly, the method is most convenient for the setting up of deletion-bearing stocks without further testing.

The method for setting up and subsequent maintenance of *'d se'*-deletion stocks is shown in figure 2. Line (1) of

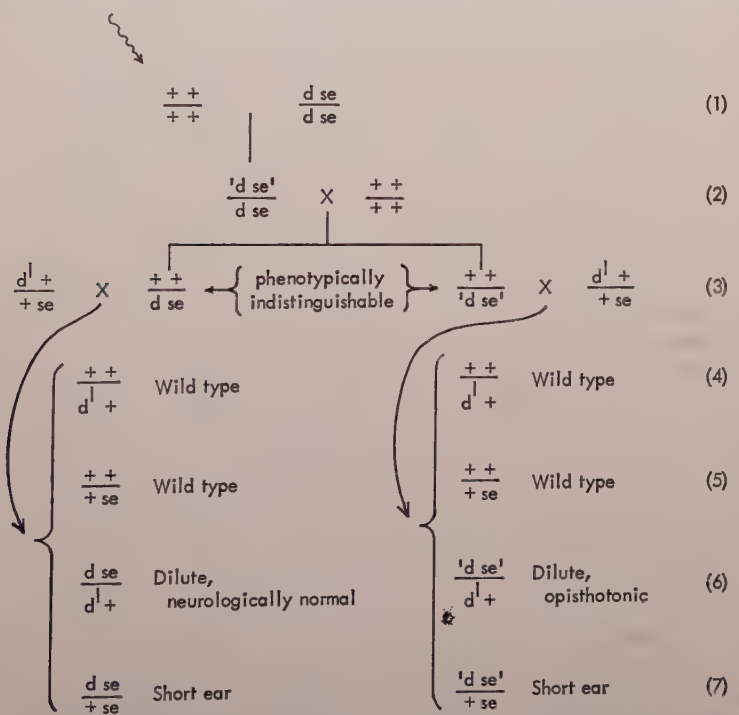

Fig. 1 Method of testing transmission of *'d se'* deletions from *'d se'*/*d se* mutants. Line (1)shows origin of mutant from irradiated wild-type (101 × C3H)F_1 and test stock animal homozygous for *d* and *se* markers. Line (2) shows mutant outcrossed to (101 × C3H)F_1 to produce large numbers of progeny. Line (3) shows test cross with animals heterozygous for d^1. The progeny from this test cross are shown in lines (4–7), with the diagnostic type in line (6).

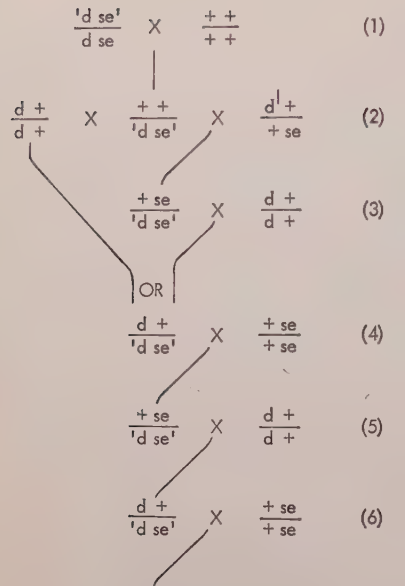

Fig. 2 Method for setting up of *d se*-deletion stock and subsequent maintenance by alternate outcrosses to *d/d* and *se/se*. This method eliminates necessity of testing for the presence of the deletion. (Note: line (1) of figure 2 corresponds to line (2) of figure 1.)

figure 2 is equivalent to line (2) of figure 1. Stocks are set up either from the short-eared progeny of animals tested to be *++/'d se'* (see fig. 1, line 7) and unrelated *d+/d+* mates (fig. 2, line 3); or, alternatively, the tested *++/'d se'* animals (fig. 2, line 2) are crossed to unrelated *d+/d+*. The dilute offspring from either of these matings are outcrossed to *+se/+se* (fig. 2, line 4); the short-eared progeny of the following generation are outcrossed to *d+/d+* (fig. 2, line 5); and so on, with alternate outcrossing to *+se/+se* and *d+/d+*. This eliminates the necessity of testing for the presence of the *d se* deletion, since each deletion-heterozygote is phenotypically recognizable.

D. *Segregation of 'd se' in various crosses.* To determine whether there are disturbances in the transmission of the deletions, one may thus examine a variety of different crosses in which *'d se'* is segregating. The one already discussed (see also table 2, "% *'d se'*") is the segregation of *'d se'* from *d se* in the original mutants (fig. 1, line 2), as determined by the relative proportions of their *++/'d se'* ar *++/d se* offspring, identified by test. Th progeny from this test cross (fig. 1, lin 3) provides a second way of measurin transmission of the deletions (fig. 1, line 4 and 5 vs. lines 6 and 7): here *'d se'* segregating from *++*. For this segreg tion, an excellent control exists in th progeny of *++/d se* littermates of th *++/'d se'* animals, which are of cours breeding at the same time and in a com pletely comparable manner (these result shown in tables 3–5, will be discusse below). Finally, the proportion of *'d s* can be determined in matings made fo stock maintenance (fig. 2, from line 3 on where segregation is from *+se* or *d+* i alternate generations. Here, however, n controls are available and the results fo litter size, in particular, must be inte preted with caution.

Segregation of *'d se'* in all the crosse (except the first) listed in the precedin paragraph is shown in tables 3–5. Th various stocks are grouped into thes tables on the basis of degree of *'d se'* trans mission from the original *'d se'/d se* mu tant animal. Thus tables 3, 4, and 5 con tain data for stocks derived from mu tants classified as *+++*, *++*, and *+*, re spectively, in table 2 (last column).

As is obvious from table 3, the variou crosses in stocks 43 CoS and 17 Z_b indicat that *'d se'* in these stocks is fully trans mitted, as was expected on the basis o the original mutants (table 2). Compari sons of litter sizes, where controls ar available, indicate no reduction in eithe male or female transmitters. The trans mission results for 130G are somewha ambiguous, especially since, owing to th early death of the original mutant, th comparison in cross (A) (see footnote t table 3), the one cross where good con trols are normally available, could not b made. However, it appears on the basi of crosses (C) and (D), that the *'d se'* i this stock should perhaps be classified a having slightly depressed transmission comparable to 99G and 209G, shown i table 4.

Table 4 contains data for the two stock set up from mutants classified as having slightly depressed transmissions. As i apparent from the table, the subsequen

TABLE 3

Transmission of 'd se' in various crosses, in stocks set up from fully transmitting mutants

Mutant stock	Cross[a]	Male parent						Female parent					
		'd se' parent			*d se* parent			*'d se'* parent			*d se* parent		
		Mean litter size	No. classif.	% *'d se'*	Mean litter size	No. classif.	% *d se*	Mean litter size	No. classif.	% *'d se'*	Mean litter size	No. classif.	% *d se*
43 CoS	(A)	6.5	180	57.2	6.5	162	55.6	5.7	86	59.3	4.7	81	56.8
	(B)	5.6	75	52.0				4.6	118	42.4			
	(C)	6.9	34	44.1				7.0	81	58.0			
17 Z_b	(A)	5.5	101	52.5	5.7	181	54.7	6.5	149	43.0	7.0	174	48.9
	(B)	5.8	56	58.9				5.5	132	58.3			
	(C)	6.7	42	54.8				6.4	122	52.5			
130 G	(A)	7.6	43	53.5	—	—	—	(7)	7	(42.9)	7.0	34	55.9
	(C)	5.6	234	40.6				5.7	270	54.4			
	(D)	6.6	372	39.8				4.5	153	35.3			

[a] Crosses from which mean litter size and progeny are shown in the body of the table are as follows:
(A) the cross shown in figure 1, line 3; i.e., segregation in $++/$*'d se'* or $++/$*d se* parent;
(B) cross of $++/$*'d se'* (F_1 of original mutant) with $+se/+se$ or $d+/d+$; i.e., segregation in $++/$*'d se'* parent;
(C) the cross shown in figure 2, line 3, 5, etc.; i.e., segregation in $+se/$*'d se'* parent;
(D) the cross shown in figure 2, lines 4, 6, etc.; i.e., segregation in $d+/$*'d se'* parent.

TABLE 4

Transmission of 'd se' in various crosses, in stocks set up from mutants having slightly depressed transmission

Mutant stock	Cross[a]	Male parent						Female parent					
		'd se' parent			*d se* parent			*'d se'* parent			*d se* parent		
		Mean litter size	No. classif.	% *'d se'*	Mean litter size	No. classif.	% *d se*	Mean litter size	No. classif.	% *'d se'*	Mean litter size	No. classif.	% *d se*
99 G	(A)	5.7	49	36.7	5.8	145	50.3	4.7	92	43.5	6.1	121	48.8
	(B)							5.0	77	40.3			
	(C)	6.4	131	36.6				4.6	333	35.7			
	(D)	5.0	225	36.4				5.6	227	39.2			
209 G	(A)	7.0	156	45.5	6.4	278	49.6	5.2	186	22.0	7.6	195	49.2
	(B)	5.6	223	40.8				4.6	131	37.4			
	(C)	5.4	59	28.8				4.8	73	52.1			
	(D)	5.5	119	45.4				5.5	69	52.2			

[a] Crosses from which mean litter size and progeny are shown in the body of the table are described in the footnote to table 3.

crosses support the original conclusi
Moreover, the litter-size data are exac
as expected for reduced transmission (
sections IA and IB): litter size of fema
is depressed to some extent, as proport
of *'d se'* progeny is reduced, but litter s
of males is not. By the criteria outlin
in section IA, this result supports the ic
of a gamete effect versus a dominant s
vital effect, an idea proposed on the ba
of the progeny of the whole array
original *'d se'/d se* mutants (see sect.
table 2).

Table 5 contains data for stocks set
from mutants classified as giving very l
transmission. Unfortunately, the resu
here are less definite, since, as a con
quence of the low transmission, only
small number of animals were availal
to work with. In the case of 201G, t
'd se' transmission of 7.1% in cross (A)
in good agreement with the value of 9.5
for the original mutant (table 2), and t
litter size is very low, as expected for
male transmitters. For the other t
stocks, however, percentage *'d se'* is high
in all the crosses listed in table 5 than
was for the original mutants. The diff
ences are not significant in the case
2CoS if one allows for the possibly high
transmission from the original mutant,
explained in footnote c to table 2. In t
case of 8 Z_b, however, the summed resu
for crosses (A) and (B) (both sexe
yield a percentage of *'d se'* (50.0%) th
is significantly higher than that for t
original mutant (12.9%). This result ca
not easily be explained in terms of
ability of the *'d se'* heterozygotes in t
different crosses, since *"% 'd se'"* in cr
(A) is based in part on what may
expected to be the least viable combi
tion, $d^1+/$*'d se'*, whereas, for the origi
mutant, it is based on the presumal
most viable combination, *++/'d se'*.
may perhaps be assumed that, in the ca
of 8 Z_b, there was, in the original muta
a gamete-lethal factor linked to *'d se';* a
that *'d se'* could therefore be transmitt
only in the few gametes that had lost th
lethal as a result of crossing over. Tra
mission from *'d se'* heterozygotes of t
subsequent generations would then be c

TABLE 5

Transmission of 'd se' in various crosses, in stocks set up from poorly transmitting mutants

Mutant stock	Cross[a]	Male parent						Female parent					
		'd se' parent			*d se* parent			*'d se'* parent			*d se* parent		
		Mean litter size	No. classif.	% *'d se'*	Mean litter size	No. classif.	% *d se*	Mean litter size	No. classif.	% *'d se'*	Mean litter size	No. classif.	% *d se*
$8\ Z_b$	(A)	6.0	11	45.5	6.6	218	49.5	3.1	13	46.1	7.7	174	44.3
	(B)	5.0	24	58.3				3.2	34	47.1			
	(D)	5.0	35	28.6				4.2	54	22.9			
2 CoS	(A)	—	—	—	4.8	143	50.4	5.4	40	27.5	7.9	134	40.3
	(C)							4.2	101	45.5			
	(D)	5.5	224	35.3									
	(E)							4.4	34	32.4			
201 G	(A)	—	—	—	6.1	467	49.7	2.6	14	7.1	7.7	163	44.2
	(F)				5.8	138	49.3				6.5	15	60.0

[a] Crosses from which mean litter size and progeny are shown in the body of the table are as follows:
(A)–(D) See footnote to table 3;
(E) Cross of $d+/$*'d se'* with $d^1+/+$*se;*
(F) Crosses of $++/$*d se* (F_1 of original mutant 201 G) with $d+/$*'d se'* from stocks 130 G or 209 G.

termined only by *'d se'* and would, presumably, be considerably higher than from the original mutant. Thus the transmission data for the 8 Z_b stock (table 5) are not dissimilar to data for the stocks derived from mutants classified as giving ++ transmission (table 4).

E. *Conclusion.* The results described in section I are, in general, in keeping with the conclusion that animals bearing *'d se'* deletions transmit these to their progeny with frequencies, in some cases, of less than the expected 50%. Although a few of the deletions may possibly have some dominant effect on viability (in view of their effect on size, see sect. IV), it is tentatively concluded, on the basis of the evidence outlined in this section, that reduction in the proportion of deletion-bearing progeny is at least partly due to an adverse effect of the deletion on the gametes.

This conclusion has two important implications. (1) According to classical theory, in animals, the genetic constitution of gametes has no effect on their survival and function. Thus even the very aneuploid meiotic products of reciprocal translocations can successfully effect fertilization. So far, the case of the *t* locus (see, e.g., Dunn and Schoenheimer, '39; Braden, '58, and this symposium) has been the only exception to classical theory, in mammals. The present data may add another such exception. It is, perhaps, possible that there are certain specific regions of the chromosome complement that are capable of affecting the function of the gamete carrying them. (2) Since the proportion of *'d se'*-bearing progeny is reduced even when the *'d se'* parent is a female, it follows (on the above assumption of a gamete effect) that the short interval between the first meiotic division of the oocyte and sperm entry may be sufficient for *d se* deletions to affect survival. Although it seems unlikely that the actual damage could express itself during this short interval, it is not inconceivable that some deleterious effect could be triggered off then and express itself at or after fertilization. (Some preliminary data indicate that this may not be until after the two-cell stage.)

II. MECHANISMS FOR THE ORIGIN OF PRESUMED *'d se'* MUTANTS

In all of the preceding discussio *'d se'* 's have been assumed to be the resu of deletions that include the *d* and *se* lo It is now necessary to consider wheth presumed *'d se'/d se* mutant animals o tained in the course of specific locus e periments could be the result of genet events other than deletions. Such consi erations become especially important i connection with a class of *'d se'/d se* an mals that has not yet been discusse namely, those giving "zero transmissio by the tests employed (table 2).

A. *The "zero-transmitting" mutant* The results for the "zero-transmitting class of mutant animals must be examine in more detail before considering possibl mechanisms for the origin of presume *'d se'/d se*. Mutants 28 FRO_4, 39 FUTh and 17 BS were tested in the manner out lined in figure 1 and discussed in sectio IC for other mutants. Although large num bers of F_1's were tested (table 6), not on was found that produced opisthotonic prog eny (among 984 and 1024 classified off spring from the F_1's of 28 FRO_4 and 3 FUTh, respectively). A majority of the F_1' were then retested either with the *se* mu tant 207K, which is homozygous lethal, o with *'d se'* stocks (particularly 99G, 130G 209G) that had already been proved to b homozygous lethal and to give lethal com binations with lethal *'se'* 's and with trans mitted *'d se'* 's of independent origin (see sect. IV and tables 8, 9). In every case viable short-eared animals were produced in about the expected proportion (table 6) The various tests here outlined were per formed for "zero-transmitting" mutants 3 FUTh, 28 FRO_4, and 17 BS, but not for 204K and 60PB, which occurred very early in our experiments. Instead, 30 and 2 lines, respectively, were set up which, with the mating scheme used, had to be homozygous either for *d se* or for *'d se'*. From mutant 39 FUTh also (which had already been tested in other ways), 12 such lines were set up. Every one of these 62 lines was viable and fertile.

Thus the "zero-transmitting" mutants seem to behave in a manner consistent

TABLE 6

Results of various crosses testing the F_1 of those presumed d se mutants which appear not to transmit d se deletions. (28 FRO_d, 39 FUTh, and 17 BS classified as "zero-transmitting"; 23 Z_b classified as "?" because of insufficient number of F_1 tested to date)

Test mate			Mutant stock tested[a]							
			28 FRO_d		39 FUTh		17 BS		23 Z_b	
Genotype	Stock		♂	♀	♂	♀	♂	♀	♂	♀
d^1+/+ *se*	101 G	No. tested	26[b]	32[c]	22	48[d]	15	11	5[e]	1[e]
		Mean litter size	6.6	6.4	5.9	7.2	6.5	5.9	6.5	6.6
		Progeny:								
		d^1+/*d se*	160	87	142	129	75	41	23	8
		d^1+/++ or + *se*/++	337	157	273	213	123	44	43	15
		+ *se*/*d se*	166	77	154	95	77	25	35	9
+se^{pl}/*d*+	207 K	No. tested	—	—	—	32[f]	—	—	—	—
		Mean litter size	—	—	—	5.8	—	—	—	—
		Progeny:								
		+ se^{pl}/*d se*	—	—	—	70	—	—	—	—
		+ se^{pl}/++ or *d* +/++	—	—	—	139	—	—	—	—
		d +/*d se*	—	—	—	73	—	—	—	—
'*d se*'/*d*+	99 G, 130 G, or 209 G	No. tested	34[b]	32[c]	—	7[e]	—	—	5[e]	1[e]
		Mean litter size	5.4	6.5	—	7.3	—	—	5.2	5.5
		Progeny:								
		'*d se*'/*d se*	122	116	—	17	—	—	30	2
		'*d se*'/++ or *d* +/++	241	196	—	54	—	—	71	9
		d +/*d se*	117	114	—	29	—	—	32	11

[a] Animals tested are F_1 of original mutant and ++/++. Their genotype is presumably ++/*d se*.
[b] Of these, 24 tested *both* with d^1 +/+ *se* and '*d se*'/*d* +.
[c] Of these, 22 tested *both* with d^1 +/+ *se* and '*d se*'/*d* +.
[d] Of these, 7 tested *both* with d^1 +/+ *se* and '*d se*'/*d*+; 32 tested *both* with d^1 +/+ *se* and + se^{pl}/*d* +.
[e] All tested *both* with d^1 +/+ *se* and '*d se*'/*d* +.
[f] All tested *both* with d^1 +/+ *se* and + se^{pl}/*d* +.

with one of the following explanations: (1) *'d se'* is completely indistinguishable from *d se;* or (2) *'d se'* is completely gamete lethal; or (3) the presumed mutant animals do not carry a mutant *'d se'* at all. These alternatives must be considered when examining the various possible mechanisms for the origin of presumed *'d se'/d se*. It may also be noted here that four of the five mutants in the "zero-transmitting" category were controls, and that, in fact, all control mutants are in this category. It would be of interest to determine whether the two spontaneous occurrences of *d se* reported by Carter, Lyon, and Phillips ('58) also fit the pattern of "zero transmission."

B. *Consideration of various mechanisms.* Among possible mechanisms, the origin of *'d se'* 's from independent simultaneous mutations at the *d* and *se* loci can, in all probability, be ruled out on the basis of frequency (see sect. III). For the "zero-transmitting" *'d se'* 's, moreover, both of the simultaneous mutations would have to be homozygous viable, which compounds the improbability since the majority of *d*-locus mutants have been of the d^1 type.

Another mechanism that must be considered is that of a mutation or deletion near, but not actually at, the *d* or *se* loci inactivating the wild-type alleles at these loci and thus leading to pseudodominant expression of the test-stock *d se*, in a manner analogous to *Notch, fa, fa*^{n}, and *spl* in *Drosophila* (Welshons, '58). Such a mechanism is not implausible for the transmitting *'d se'* 's. If it exists, it should lead to crossovers between the inactivator and either *d* or *se*, but, with one exception, the various matings made for testing and stock maintenance (fig. 1, 2) would not detect such crossovers. The exception is this: if a crossover between the inactivator and *d* occurred in the original mutant (presumed *'d se'/d se*) animal, it would, with a probability of 0.5, lead to an F_1 individual that failed to produce dilute progeny in the test mating with $d^1+/+se$. The fact that no such F_1's have yet been found in a total of about 300 tested in the various transmitting stocks has, however, little meaning, since the probability of the events outlined is very small. Special crosses are being set up now to detect the presence of a possible inactivator of *d* a *se* in the transmitting *'d se'* stocks. W respect to the "zero-transmitting" stocks is difficult to conceive of an "inactivat since one would have to postulate that effect was to change the action of both and $+^{se}$ to the exact level of the test st alleles *d* and *se*, rather than to inactiv them completely.

Another mechanism to be considered loss of the chromosome 2 that is deriv from the wild-type parent. On this hy thesis, presumed *d se/'d se'* animals a in reality, *d se/O*. If the O gamete of *d se/O* parent is viable to any extent, o could get results compatible with those d cussed for some of the transmitted *'d se'* There are, however, serious objections this hypothesis. To begin with, it see unlikely that monosomy for an autoso would be compatible with viability. F thermore, there are two specific indicatio that not all the transmitting *d se* muta can be of the *d se/O* type: (1) transm sion from the various mutants is quite d ferent (see sect. I); and (2) chromoso counts from a bone marrow squash pre ration (kindly made by Dr. Welshons, the method of Welshons and Russell, '5 in 209G, one of the eight transmitti stocks, revealed 40 chromosomes. Th it must be concluded that the *d se/O* terpretation does not apply to at least so of the transmitting *d se* mutants and m of course, not apply to any of them.

As far as the "zero-transmitting" m tants are concerned, the *d se/O* hypothe seems, at first glance, an attractive o since, by extension of an earlier argume (sect. IE), the *O* gamete might be n functional. On this hypothesis, howev female *d se/O* mutants should have o half-normal litter size (table 1) and t is obviously not the case for 39 FUTh, PB, and 17 BS (see table 2). No conc sion can be drawn for male mutants, whi could have normal litter size in any c (table 1 and sect. IA). Other conside tions, too, support the conclusion that t "zero-transmission" category is not mer the extreme end of a progression goi from full transmission, through reduc transmission, to no transmission. One these is size of the presumed mutant a mal. All the mutants that gave reduc

ransmission were of distinctly small size, ompared with littermates. The "zero-ransmitting" presumed mutants, on the ther hand, were either of normal size or of nly slightly reduced size. (Animals homo-ygous for the test-stock marker *se* are ften somewhat small; see also Law, '38.) nother point to be noted is that 39 FUTh ame from irradiated oocytes. If it was, in-eed, induced by irradiation (rather than a pontaneous mutation occurring in this roup), this would rule out complete gam-te lethality, for (as shown in sect. IA), if gamete-lethal '*d se*' "mutation" had been nduced, the mutant animal herself could ot have been conceived. Finally, one of he "zero-transmitting" mutants, 17BS, ave a chromosome count of 40 in a tail bi-psy culture (kindly prepared by Dr. Chu). n the basis of all these various considera-ions and findings, the *d se/O* hypothesis, herefore, seems as untenable for the zero-transmitting" class as it did for at east some of the transmitting *d se* mu-ants.

The hypothesis that best fits the "zero-ransmitting" class is that these animals re homozygous for the test-stock *d se* as a esult of double nondisjunction. This ould explain the facts that they transmit nly *d se* indistinguishable from the test-tock markers, yield normal litter size, and re of relatively normal size. It may be oted that in the cross of a homozygous ild-type animal with a homozygous *d se* est-stock animal, such as is made in our pecific mutation rate experiments, nondis-unction of chromosome 2 in one or both arents can lead to 8 possible types, of hich 6 are presumably either lethal or ild type and, therefore, not detectable. he remaining two are: first, the mono-omic *d se* resulting from nondisjunction n the wild-type parent only, a situation hat has been discussed; and, second, the *se* homozygote with both of its chromo-omes 2 derived from the test stock, which esults from nondisjunction in both par-nts. It might seem, at first glance, that ondisjunction in *both* parents would be oo rare an event to be considered, espe-ially since the required *d se/d se* type rep-esents only one of 4 possible combinations esulting from such double nondisjunction. owever, the percentage incidence of zero-transmitting *d se* "mutants" is quite consistent with the frequency of double nondisjunction that would be expected from a rate of single nondisjunction such as has actually been found for the sex chromosome (Russell, Russell, and Gower, '59; Welshons and Russell, '59). On the other hand, it appears from various lines of evidence, which will be discussed in a future publication, that the frequency of recovered cases of double nondisjunction of the 4 other autosomes marked in our specific locus experiments cannot be as high as it would seem to be, on the above hypothesis, for chromosome 2. There might be a variety of reasons for this; e.g., our random-bred multiple recessive test stock might carry a higher frequency of lethals on these 4 autosomes than on chromosome 2. Chromosome-2 nondisjunctions should, of course, produce the *d se/O* type in considerably larger numbers than *d se/d se* and, if the present indications that this type is not represented among the mutants can be verified, it would indicate that *d se/O* is lethal.

C. *Conclusions.* A number of mechanisms that might produce presumed *d se* mutants have been considered, namely: deletion, simultaneous mutation of *d* and *se*, inactivation of *d* and *se* through position effect, nondisjunction in the wild-type parent producing the monosomic *d se/O*, and nondisjunction in both parents leading to an individual homozygous for the test-stock *d se*. The transmitting mutants are best explained on the basis of deletions of a region involving $+^{d}$ and $+^{se}$. However, position-effect-inactivation is still a possible explanation that must be tested in further experiments. For the "zero-transmitting" mutants, the most plausible hypothesis at this time is that they are, in effect, homozygous for the test-stock chromosome 2 as a result of nondisjunction in both parents.

III. RELATION BETWEEN GERM CELL STAGE AND TYPE OF GENETIC CHANGE INDUCED

Table 7 shows the relative numbers of *d*, *se*, and *d se* mutants that have been obtained at this laboratory from the irradiation of various germ cell stages and in controls. Since, in all our experiments, the three events were always scored simultane-

TABLE 7

Relative numbers of d, se, and d se mutations obtained at this laboratory from the irradiation of various germ cell stages and in controls

Stage	*'d'*	*'se'*	*'d se'*
Spermatogonia	39	7	1
Postspermatogonial	6	5	6
Oocytes	1	0	4
Control males	8	0	4
Control females	0	1	0

ously, comparisons of the relative frequencies of the three events are valid. However, comparison of the total frequencies in the different rows of this particular table would be meaningless, because there are large differences in the numbers of animals obtained from the various irradiated germ cell stages; and the results for each stage, as a matter of fact, represent combinations of experiments with different doses and dose rates. (Other publications may be consulted for the actual mutation rates at various loci; e.g., Russell and Russell, '59; Russell, Russell, and Cupp, '59; Russell, Bangham, and Gower, '58.)

As has already been mentioned in the Introduction, the yield of *d se* mutants from spermatogonial irradiation is no higher than control frequency. Of the two alternative explanations—either that such deletions are not induced in spermatogonia, or that they are induced but not transmitted through the gametic phase—the latter was discarded since we were able to show that *d se* deletions are transmissible, although sometimes to a reduced extent. Because transmission may be reduced, it is not inconceivable that the probability of recovering *'d se'* induced in spermatogonia may be lower than it is for other mutations. However, the factor is presumably not large (the average degree of transmission for the 8 transmitting mutants tested was about two-thirds of normal, see table 2), and even if one allows for it, it is clear that if *'d se'* deletions are induced at all in spermatogonia, it must be with a very low frequency. It may be recalled that the only *d se* mutant that was derived from irradiated spermatogonial stages, 43CoS, transmitted the mutant chromosome without reduction in frequency. If 43CoS was indeed the result of irradiation, rather than of spontaneous o currence, it might indicate that, in th rare instances when deletions are induce in spermatogonia, these are small ones.

In contrast to spermatogonia, postspe matogonial stages and oocytes yield a rel tively high frequency of induced deletion As may be recalled from section I B, se eral of these are transmitted to a *reduce* extent and may therefore be assumed t represent longer deficiencies.

Finally, as was shown in section II, th nature of the control *presumed d se* mu tants may be entirely different from tha of the induced ones. Instead of carrying deficient chromosome, they may be homo zygous for the test-stock *d se* as a result o nondisjunction in both parents. It wa also mentioned that the spontaneous non disjunction frequency for the other fou autosomes marked in our experiment could not be as high as the frequency fo chromosome 2. Even so, if the nondis junction hypothesis for the origin of spon taneous *'d se'* mutants should turn out t be correct, it would indicate that a class o spontaneous "mutations," which whe first detected, might be assumed to be iden tical with a similar-appearing class of radi ation-induced mutations, may, on furthe analysis, turn out to be of an entirely dif ferent qualitative nature.

IV. THE FINER STRUCTURE OF THE *d se* REGION

The large number of independent mu tations and deletions that are now avail able in the *d-se* region (table 7) open th way for detailed genetic analyses of tha region. A high proportion of the *d-* and *se* locus mutations have been tested with re spect to viability of the homozygote, a have seven of the *d se* deletions. In addi tion, almost all the possible combination of *'d se'* versus *'d se'*, and *'d se'* versus *'se* mutants have been made, as well as sev eral selected combinations of *'d se'* versu *'d'*, *'d'* versus *'d'*, and *'d'* versus *'se'*. Thes will be discussed later in this section.

Of 54 independent *d*-locus mutations s far obtained (table 7), 37 have been mad homozygous. Of these, 33 turned out to b of the d^t type (opisthotonic), 2 are pre natally lethal (these will be referred to a d^{pl}), and 2 are homozygous viable and in

ermediate in coat color between d/d and ild type. The question arises whether d^1 ight be a pseudoallele of d. This ques-on can be settled only if it is shown that ne frequency of wild-type offspring from $^1/d$ parents exceeds the reverse mutation ate of d and of d^1. (The spontaneous rate f $d \rightarrow +$ is presumably very low: no re-ersion of d has yet been observed in about 50,000 animals of our multiple recessive est stock.) One wild-type offspring was btained in a cross of $d^1+/d\ se \times d^1+/d\ se$ nd was tested to be $d^1+/+se$, thus appar-ntly representing a reversion of d. On he hypothesis of pseudoallelism, this event ould represent a crossover and indicate hat d^1 was situated *between* d and *se*.

Since, in all cases tested, the combina-ion of d^1 with d *se* deletions leads to a phe-otype indistinguishable from d^1/d^1 (i.e., ilute fur; convulsions with opisthotonus), may be concluded that d^1 is an amorph nd could be a small deletion. The fact hat d also appears to be an amorph ($d+/$ *l se'* is no lighter in color than d/d) would eem to lend support to the idea that d and ' are pseudoalleles rather than true alleles. n interesting finding is that d^1/d^{p1} has a henotype indistinguishable from d^1/d^1. his may indicate that d^{p1} is a dele-ion: if d^1 is a true allele of d, then d^{p1} may nclude some adjacent region whose pres-nce is essential for postnatal survival; if, n the other hand, d^1 is a pseudoallele of , d^{p1} presumably includes a region adja-ent to d^1, which may—but need not—con-ain the d "locus" itself. It is noteworthy hat neither of the two d^{p1} mutants came rom irradiated spermatogonia: one was spontaneous and the other came from ir-radiated oocytes (which, it may be re-called, have also yielded *d se* deletions).

Of the 13 independent *se* mutations, 12 have been obtained in homozygous condi-tion and all but two of these are viable. In the two cases (both derived from irradi-ated spermatogonia) where homozygosis is lethal, death occurs prenatally. These mu-tants are provisionally designated se^{p1}. One of them has been tested with d^{p1} and the combination of $d^{p1}+/+se^{p1}$ turned out to be viable and normal in every way, indicating that the region responsible for lethality in the two cases is not the same.

Seven of the *d se* deletions (namely, all the transmitted ones except 201G) have been at least partially tested for the effects in homozygous condition. In no case has a viable homozygote been obtained (table 8). (It should be pointed out that the data are not yet adequate for $8Z_b$ and 2CoS, where the expected frequency of homozygotes is low because of reduced transmission.) Furthermore, no case in which *'d se'*'s have been adequately tested against each other has yielded a viable combination (table 8). The simplest as-sumption is that the various independently induced deletions lack one or more *com-mon* vital regions.

Results of tests of the *d se* deletions against the bulk of the *se* mutants and against some of the *d* mutants are shown in table 9. Without going into the details of this checkerboard, it is obvious that the various *'d se'*'s in combination with any viable *se* mutant yield viable short-eared young. It should be noted here that these

TABLE 8

Evidence for the lethality of combinations of d se deletions of like and different origin[a]

Stock	43 CoS	17 Z_b	130 G	99 G	209 G	8 Z_b	2 CoS
43 CoS	67:0	61:0	47:0	44:0	52:0	42:0	43:0
17 Z_b	—	78:0	39:0	43:0	89:0	95:0	93:0
130 G	—	—	129:0	105:0	121:0	38:0	42:0
99 G	—	—	—	330:0	79:0	35:0	32:0
209 G	—	—	—	—	96:0	—	83:0
8 Z_b	—	—	—	—	—	29:0	—
2 CoS	—	—	—	—	—	—	37:0

[a] Each pair of figures separated by a colon represents the number of non-*'d se'/'d se'* and *'d se'/'d se'*, respectively. If any of the *'d se'/'d se'* combinations had been viable, the expected ratio would have been 3:1 where transmission is normal. (Where transmission is less than normal, the expected frequency of *'d se'/'d se'* would range from something less than ¼ to possibly considerably less. See tables 2–5 and text for information on degree of transmission.)

TABLE 9

Results of crossing various d se deletions to various independent se and d mutations[a]

d- or *se*-locus mutation		*d se* deletion						
Type	Symbol	43 CoS	17 Z_b	130 G	99 G	209 G	8 Z_b	2 CoS
'se'	23 M[b]	77:*69*	78:*110*	262:242	281:*171*	66:*69*	41:*11*	122:*79*
'se'	14 ThP[b]	14:*9*	19:*20*	13:*5*	16:*7*	9:*4*	—	26:*14*
'se'	10 DT[b]	37:*43*	42:*33*	—	17:*8*	33:*23*	—	—
'se'	8 CoS[b]	34:*18*	59:*53*	14:*18*	30:*26*	8:*1*	—	—
se[pl]	52 CoS[b]	14:38:*0*	12:37:*0*	13:29:*0*	23:38:*0*	—	15:11:*0*	13:19:*0*
se[pl]	207 K[b]	20:52:*0*	20:43:*0*	10:35:*0*	30:57:*0*	34:68:*0*	11:19:*0*	—
'se'	193 G[c]	—	25:*21*	11:16:*14*	7:21:*9*	16:29:*18*	—	—
'se'	196 G[c]	12:*17*	—	30:46:*25*	39:77:*30*	13:31:*11*	—	—
'se'	1 DT[c]	36:*56*	29:*31*	29:*24*	66:*50*	94:*76*	22:*3*	14:*12*
'se'	1 CoS[c]	36:*37*	18:*17*	34:*37*	37:*28*	20:*16*	—	—
'se'	3 CoS[c]	31:*28*	51:*56*	31:*19*	62:*36*	72:*61*	—	15:*3*
d^l	123 G[b]		3:5:7[e]					
d^l	155 G[b]		3:12:3[e]					
d^l	205 K[b]			8:10:3[e]	7:10:2[e]	18:36:20[e]		
d^l	5 XY[b]			16:28:5[e]	12:39:*12*[e]	2:3:*1*[e]		
d^l	101 G[c]	66:124:*69*[e]	48:116:*53*[e]	8:14:7[e]	7:26:*10*[e]	72:212:*58*[e]	8:29:3[e]	4:13:7[e]
d^l	208 K[d]			15:29:*17*[e]	2:8:6[e]	15:26:*18*[e]		
d^{pl}	2 G[d]	20:29:*0*	11:10:9:*0*	8:5:10:*0*	18:17:13:*0*	3:3:1:*0*	10:7:4:*0*	16:16:*0*

[a] Numbers in italics represent segregants that carry the combination of *'d se'* and *+'se'* or *'d'+*, respectively. The expected ratios (assuming complete transmission—see, however, tables 2–5) are as follows: where two figures are given, 1:1 (crosses of type *+'se'/+ 'se'* × *d +/'d se'*); where three figures are given, 1:2:1 (crosses of type *+ 'se'/d +* × *d +/'d se'* for *se* mutations, *d^l +/+ se* × *++/'d se'* for *d^l* mutations, *d^{pl} +/+ se* × *+ se/'d se'* for *d^{pl}* combinations); where four figures are given, 1:1:1:1 (crosses of type *d^{pl} +/d se* × *+se/'d se'*).

[b] Derived from irradiated spermatogonia.

[c] Derived from irradiated postspermatogonial stage.

[d] Spontaneous.

[e] Opisthotonic dilute.

hort-eared young are often of reduced ody size. In general, the size reduction is reatest in combinations involving *'d se'* 's lassified as having + transmission, and nly moderate or barely detectable in com- inations involving +++-transmitting *'d e'* 's. All combinations of *'d se'* 's with ethal *se* mutants (i.e., se^{pl} by the desig- ation used) are prenatally lethal. The ame is true of all the combinations of *d se'* 's with d^{pl}. These findings, added to he ones discussed earlier in this section particularly the nature of the $d^{pl}+/+se^{pl}$ nd the d^{pl}/d^{l} combinations), indicate that ll seven of the tested *d se* deletions lack at east two vital regions: one closely associ- ted with the *se* locus, the other with the *d* ocus. It is not *necessary* to assume a greater length of deletion than one that would just include *d* and *se;* although it seems likely—on the basis of such factors as the differences in transmission, that have been demonstrated among the various *d se'* 's—that the extent of at least some of the deletions is greater than this minimum length.

SUMMARY

1. Animals that are mutant for both *d* and *se*, two closely linked loci, have been recovered after irradiation of postspermatogonial stages and of oocytes. Of various mechanisms considered, the one that seems most plausible at this time is deletion involving the *d* and *se* loci.

2. Irradiation of spermatogonia yields such deletions only with extremely low frequency, if at all.

3. Although presumed *d se* mutants have occurred spontaneously, breeding results and other properties make it appear highly unlikely that they represent *d se* deletions. The most plausible hypothesis for these animals is that they are homozygous for the test-stock chromosome 2 as a result of nondisjunction in both parents.

4. Several of the *d se* deletions are transmitted with less than normal frequency. Present data indicate that this is due to a semilethal effect on *gametes*. If this is indeed the case, it would add another exception—similar to that of the *t* locus—to the rule that, in animals, genetic constitution of gametes has no effect on their survival and function.

5. With large numbers of independent mutations and deletions now available in the *d se* region, a detailed genetic analysis of this region has been made by producing over 50 kinds of homozygotes and by making over 100 combinations of independent mutations and deletions. These reveal the following: (a) About 90% of *d*-locus mutations are of the d^{l} type. The rest are either prenatally lethal or viable and intermediate in coat color. (b) There is a possibility, but as yet no definite proof, that d^{l} is a pseudoallele of *d*. If so, it must be situated between *d* and *se*. (c) d^{l} is an amorph, since combinations of *d se* deletions with d^{l} are indistinguishable from d^{l}/d^{l}. Similarly, *d* appears to be an amorph, lending support to the idea of a *pseudo*-allelic relation between *d* and d^{l}. (d) The prenatally lethal *d* mutations may be small deletions. (e) Over 80% of *se*-locus mutations are homozygous viable. The rest are prenatally lethal. (f) The region responsible for lethality in the case of prenatally lethal *d* and *se* mutations is not the the same. (g) All *d se* deletions are prenatally lethal in the homozygous state, in combination with each other, and in combination with prenatally lethal *d*- or *se*-locus mutations. That the extent of at least some of the deletions may, however, be greater than this minimum length is indicated by the marked differences in transmissibility among the *'d se'* 's.

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We wish to express our deep appreciation to Mrs. M. H. Steele, Miss E. M. Kelly, Mrs. J. S. Gower, and to the other members of the Mammalian Genetics and Development Section who have assisted in many ways in obtaining the data discussed in the present paper. We are also grateful to Drs. W. J. Welshons and E. H. Y. Chu for chromosome counts on two of the mutants.

OPEN DISCUSSION

SEARLE[3]: I think that there is little, if anything, in the way of specific-locus results that Harwell can contribute to this very fine study of the Russells' on simultaneous dilute short-ear mutations. But

[3] A. G. Searle, Radiobiological Research Unit, Harwell.

perhaps I ought to give our data as far as I have it available just for the record.

We have had altogether four of these simultaneous mutations, as well as 12 mutations at the dilute locus alone. But curiously enough, we have not had any at all that are short-ear alone. Out of the four dilute short-ear, one of them at least is completely viable, just as Dr. Russell found with some of theirs. I quite agree that this is very unlikely to be an actual homozygous deletion, but must be attributable to some other cause. We have not gone into the transmission frequency in detail; but in one of the mutants the transmission was apparently reduced quite considerably. We have actually been studying these mutants mainly by intercrossing after outcrosses. With the dilute mutants, we have —like Dr. Russell—had a number of dilute lethals (opisthotonics). I should think about a third of them are like that, perhaps a bit more. But we also have had a number that are lethal at an early embryonic stage, and only one or two viable.

I think that is all we can say unless Dr. Lyon, who really knows more about it, has anything to add on our results with these loci.

L. B. RUSSELL: I should like to ask what was the origin of the *d se* that acts like a viable—in other words, the one that, according to our ideas would be a double nondisjunction. Was that a control?

SEARLE: I am fairly certain it was a control, but I can't be quite definite, I am afraid.

L. B. RUSSELL: That would of course fit in very well and make the case that much better.

LYON[4]: I think that the viable dilute short-ear was almost certainly spontaneous because it was in an experiment where we were using a very low dose. So it probably was spontaneous, whatever series it was in.

GREEN[5]: Since the short-ear gene has so many other morphological effects on the phenotype, I wonder if you have observed any of the other effects typically associated with Lynch's short-ear in the radiation-induced mutations, including the bifurcated xiphisternum.

L. B. RUSSELL: No, I am sorry to say we just have not looked beyond the external appearance of the ear. It would, of course, be most valuable to carry out thorough studies of the type Dr. Margaret Green has done.

M. GREEN[6]: I would like to ask a question about the crossovers between the dilute and lethal effects of dilute lethal. You did not separate the lethal effect from the dilute effect?

L. B. RUSSELL: No. It might, perhaps, clarify the situation if I describe the circumstances of this presumed crossover. In a cross of $d^1 +/d\ se \times d^1 +/d\ se$, which should produce only dilute-colored progeny, there occurred a wild-type animal that, on test, turned out to be $d^1 +/+\ se$. Now, such an animal could, of course, be the result of the reverse mutation $d \rightarrow +$. If, however, it was the result of crossing over between pseudoalleles (and one single case, naturally, cannot prove this), its genotype would indicate that d^1 was situated between *d* and *se*. For, if the order were, instead, d^1, *d*, *se*, the genotype of the wild-type animal would have been $d^1 +/++$ *or* $d\ se/++$.

MARKERT[7]: You also mentioned in passing that there was some evidence that the *a* locus might be compound.

L. B. RUSSELL: Yes. We have a mutation called a^z, which is an intermediate allele at the *a* locus. It is homozygous lethal. The phenotype of a^z/a is pale-bellied non-Agouti. We maintain a^z in a balanced lethal stock, $A^y/a^z \times A^y/a^z$, which breeds true for yellow. In this balanced lethal stock, we have had, three or four occurrences of wild-type animals in only a few hundred young. These could be caused by crossing over between A^y and a^z, unless they represent a high reversion rate of a^z. We are now marking both sides of the locus, so we should know soon.

HEXTER[8]: In your dilute case, how do you know there is a recombination?

L. B. RUSSELL: We don't, since we carry no other markers on that chromo-

[4] M. F. Lyon, Atomic Energy Research Establishment, Harwell.

[5] E. L. Green, Roscoe B. Jackson Memorial Laboratory.

[6] M. Green, Roscoe B. Jackson Memorial Laboratory.

[7] C. L. Markert, Johns Hopkins University.

[8] Wm. H. Hexter, Amherst College.

ome. It could be a reverse mutation, too. But the fact that hundreds of thousands of dilute animals in our colony that could have reverted to wild type have not yet done so makes us think that reverse mutation at this locus is extremely rare. On the other hand, it would obviously be foolish to conclude from the *one* case I have outlined that we were dealing with crossing over. The main reason for citing it was to show that *if* there is crossing over, *d*[1] must be between *d* and *se*.

R. M. VALENCIA[9] : We (J. I. Valencia and I) have some information from our recent work that bears on the problem of whether chromosome rearrangements can be recovered from treated oogonia. We have been analyzing X-ray-induced mutations at specific loci in the *Drosophila* female. Among those recovered from treated oogonia, we have one that is a deficiency and one that is associated with a translocation between X and chromosome 2. The genetic scheme excludes the possibility that these might have been existing in the stock. If they were of spontaneous origin, they would have had to occur in the irradiated generation. Since we have made cytological examinations of a large number of spontaneous specific locus mutations and have found none of them associated with chromosome aberrations, we think it is very probable that these are radiation induced.

I rather hesitatingly mention another piece of evidence that seems to indicate the recoverability of chromosome breaks from the oogonia. In a scute[51] inverted X chromosome, the normal allele of yellow is placed in the vicinity of heterochromatin. After irradiation there is a high frequency of y^+ losses, which are attributed to chromosome rearrangements (mostly small deficiencies) involving the adjacent heterochromatin and the y^+ locus. We irradiated females heterozygous for a sc[51] In 49 sc[8] X chromosome and a normal X and looked for such losses in the two types of X treated as oocytes and as oogonia. From an examination of something more than 3000 females developed from inverted X's of irradiated oogonia, we got four yellows. This is a very high frequency—much higher than the frequency we get in the ordinary chromosome where y^+ is in its normal euchromatic location. (In this particular experiment, we found none from the normal X treated in oogonia; but from previous experiments we found, in a similar setup, three in 42,828.) If this excess in mutation frequency does in fact represent heterochromatic deficiencies, then it would appear that we are recovering deficiencies induced in the oogonia.

E. S. RUSSELL[10] : I just wanted to comment on the rate of mutation from *d* to *D*. I have had two. One occurred in DBA/1, and one occurred in DBA/2, and they have been tested in linkage, too.

HOECKER[11] : I would like to add that we had a mutation from *d* to *D* in our DBA/1 stock. This one was a somatic and gonadic mosaic. Its coat looked like a leopard and it gave about one-fourth intense progeny. Frequency would be at least one in about 150,000 mice bred.

BATEMEN[12] : May I bring some *Drosophila* in this as well? We were actually concerned with induced crossing over in males heterozygous for *b pr vg*. We thought that this was going to help us to identify more exactly which type of germ cell was being sampled on the particular days after irradiation. But as a result, as a byproduct, we got some data on this question of deletions, I think. We gave 1000 r and explored the irradiated germ cells for crossovers. You get what Auerbach calls r_1 and r_2 types of recombinants. The r_1's are those with a single recessive marker and the r_2's are those with two recessive markers. The single recessive markers could be attributable to recessive mutations or deletion. The r_2, of course, is much more likely to be true crossing over. When you are concerned with crossing over, then r_1 and r_2 will be in equal frequencies. On days 6 and 7, which I feel are probably irradiated spermatocytes, we got a small number of apparent crossovers. But there was a large excess of r_1 over r_2. Therefore it looks as though we were getting a high induced mutation rate

[9] R. M. Valencia, Argentine Atomic Energy Commission, Buenos Aires.

[10] E. S. Russell, Roscoe B. Jackson Memorial Laboratory.

[11] G. Hoecker, Instituto de Biologia "Juan Noe," Santiago.

[12] A. J. Bateman, Christie Hospital, Manchester.

in the spermatocytes. There was, of course, the possibility that there were deletions here rather than point mutations.

I was particularly hoping that they would be deletions because there is the peculiar phenomenon of the very high yield of deleted X's at this same period after irradiation. It would be nice to find some phenomenon that paralleled the sensitivity pattern of the deleted X's. We therefore tested some of these, both the r_1 and the r_2 types. In the r_1's we were looking for deletions. We were expecting the r_2's to be a sort of control; that is, they would be true crossovers.

These tests are still in a rather early state but it does look as though both the r_1 and the r_2 induced in this period act as lethals and are not true crossovers. We were looking for these crossovers or apparent crossovers right from the first matings. But it was not until this stage that we got any measurable quantity of the apparent crossovers. I might add that there is a large amount of induced crossing over from then onward (from the spermatogonia) but no apparent excess of r_1 over r_2. In other words, no apparent important contribution from mutations or deletions.

I don't suppose one could do that sort of thing in the mouse; that is, collecting mutations or apparent mutations in the spermatocytes. But it does look as though in *Drosophila*, anyway, the spermatocytes are particularly sensitive to the induction of deletions.

L. B. RUSSELL: I think your results are most interesting. Although, as you say, it is difficult, in the mouse, to get results from spermatocytes, I should like to point out the parallel between the mouse oocyte results and your *Drosophila* spermatocyte data. As you may remember, our oocytes gave relatively the highest frequencies of *d se* in comparison with separate *d* or *se* mutations. So it seems that in oocytes, too, one gets a particularly high incidence of deletions, compared to point mutations. It is also interesting that you got no deletions from irradiated spermatogonia, paralleling our mouse spermatogonia results.

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Symposium on Mammalian Genetics and Reproduction

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